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THE FIRST HUNGARIAN RECORD OF A RARE *GOMPHONEMA* (BACILLARIOPHYTA) SPECIES FROM TEMPORARY PONDS

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Abstract: The first record of *Gomphonema jadwigiae* in Hungary and morphological characteristics of the species (including scanning electron microscope details) are provided. The species was observed in samples taken from some bomb crater ponds situated in the northernmost part of the Kiskunság National Park, near the village of Apaj, in the plain of the Danube–Tisza Interfluve, Central Hungary. *Gomphonema jadwigiae* occurred in three ponds, and it was dominant in one of them. In its first description the species was regarded as an oligotrophic indicator, but our results suggest that it can tolerate the elevated salinity and nutrient concentration as well.

Key words: *Gomphonema jadwigiae*, bomb crater ponds, salinity

INTRODUCTION

The diatoms are considered to be useful indicators of environmental changes, and particularly used as indicators of organic pollution and high nutrient loads (Smol & Stoermer 2010). The accurate ecological interpretation of their autecological characteristics requires correct identification of the species. This can be particularly difficult task in the case of the genus *Gomphonema* which has great morphological variation (Levkov *et al.* 2016). They attach to the substrate with branched mucilaginous stalk secreted via apical pore field situated only on foot pole of the frustulum, which is characteristic to this genus. Many *Gomphonema* species have proved to be important indicators of environmental conditions, but taxa belonging to this genus cannot be identified using solely light microscope, because of the overlapping characteristics (Wojtal 2003). The taxa of the genus

Gomphonema are cosmopolitan, relatively common in freshwater benthic communities (Wojtal 2003), but some species can tolerate the elevated salinity as well (e.g. *G. olivaceum* var. *calcarea* (Cleve) Cleve in Van Heurck (Denys 1991); *G. salinarum* (Pantocsek) Cleve (Lange-Bertalot & Steindorf 1996).

Extreme habitats, like small, sodic bomb crater ponds, even if they are artificial ones, may host unique flora and fauna and contribute over-proportionally to regional biodiversity (Vad *et al.* 2017). We found a *Gomphonema* species identified as *Gomphonema jadwigiae* Lange-Bertalot & E. Reichardt, which is rare all over the world. The species was described from an Austrian lake near Lermoos in 1996 based on light microscopic investigation without any scanning electron micrographs. Moreover there are not any kind of type material (pers. comm. of authors) for reinvestigation. The species was dominant in bomb crater ponds at Apaj, allowing us to perform a detailed electron microscopic investigation. We have already reported its occurrence from this habitat (e.g. Ács *et al.* 2017), but without detailed morphological taxonomic description and photo documentation, which is important for e.g. the 'analysts' participating in monitoring programs, especially if a species is dominant in some water bodies.

Our aim is to provide a detailed morphological description of this dominant *Gomphonema* species using scanning electron microscope (SEM) for demonstrating its first Hungarian occurrence.

MATERIAL AND METHODS

Study site

A dense cluster of bomb crater ponds (created by mistargeted bombing of the nearby airport during World War II) is situated in the northernmost part of the Kiskunság National Park (47.12338° N, 19.13645° E), near the village of Apaj, in the plain of the Danube–Tisza Interfluve, Central Hungary in an area of approximately 25 hectares. This area has patchy surface salinization, because the flow pattern of groundwater results in extensive surface salinization in those discharge areas where the infiltrating freshwater does not superimpose the upwelling saline water (Simon *et al.* 2011). The patchy surface salinization results in different salinity of the water of bomb crater ponds here. More information of the study sites is available in Vad *et al.* (2017).

Sampling

Altogether 48 ponds were sampled for benthic diatoms. Samples were taken from 10 cm section (or maximal length if the depth of pond was less than

10 cm) of the green common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) stems, or, if it was absent, from alkali bulrush (*Bolboschoenus maritimus* (L.) Palla) or narrowleaf cattail (*Typha angustifolia* L.) between 7 and 9 May, 2014. Stems were chosen randomly in five replicates per pond. The following physico-chemical variables were measured in the case of each pond: water depth, diameter of pond, percentages of open water surface, submerged and emergent macrophyte coverage, conductivity, pH, water temperature, turbidity, total suspended solids (TSS), total phosphorous (TP), nitrate (NO_3-N), ammonium (NH_4-N), chlorophyll-*a* (Chl *a*), calcium (Ca^{2+}) and chloride (Cl^-) concentration. The determination methods and more details of sampling are available in Vad *et al.* (2017).

Microscopic investigations

The diatom frustules were cleaned with hydrochloric acid and hydrogen peroxide, subsequently washed in distilled water and mounted with Naphrax® medium (CEN 2014). An Olympus IX70 inverted microscope equipped with differential interference contrast (DIC) optics was used for LM observations, for the micrographs we used Zeiss Axio Imager Z2 light microscope equipped with Axiocam 506. For SEM studies a part of the cleaned and washed samples was filtered through a 3 μm Isopore™ polycarbonate membrane filter (Merck Millipore), which was fixed onto a stub using double-sided carbon tape, and coated with gold using a rotary-pumped spatter coater Quorum Q150R S. The fine structures of the diatom frustules were observed with Zeiss EVO MA 10 SEM operated at 10 kV and 10 mm distance using SE detector.

RESULTS

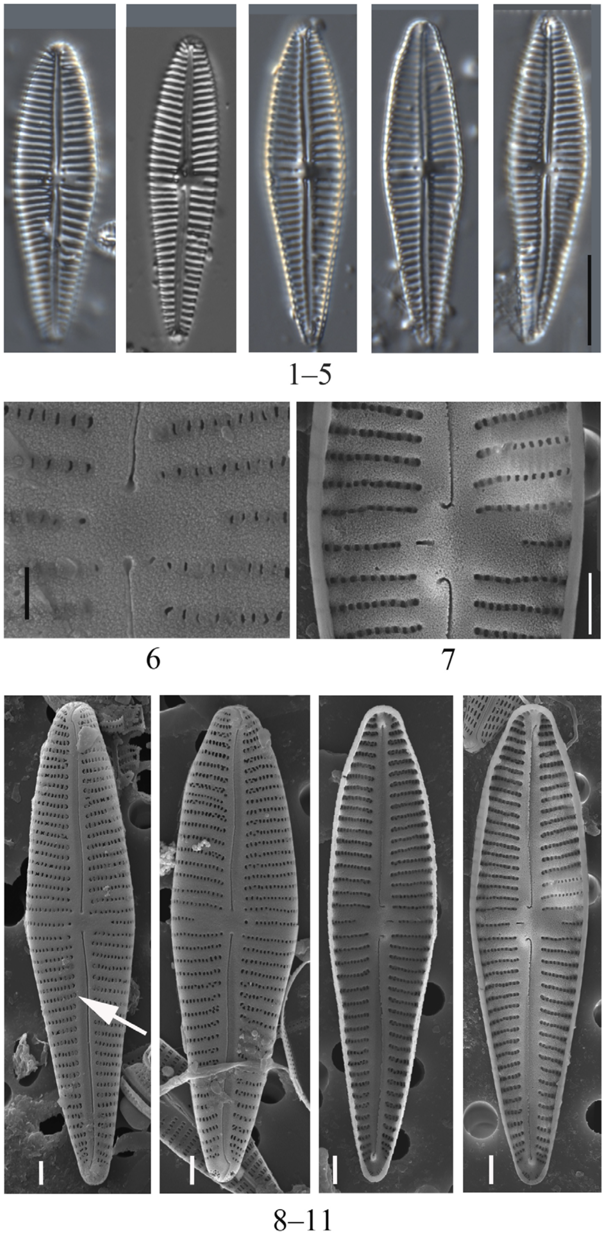
Gomphonema jadwigiae occurred only in three ponds (from the 48 studied ones) and it was dominant only in one pond (relative abundance: 8.9%). Moreover, sixteen species, including *Gomphonema angustatum* (Kützing) Rabenhorst, *G. productum* (Grunow) Lange-Bertalot & Reichardt, *Halamphora dominici* Ács & Levkov, *Navicula veneta* Kützing, *N. wiesneri* Lange-Bertalot, *Nitzschia austriaca* Hustedt, *N. reskoi* Ács, Duleba, C. E. Wetzel & Ector, *Psammodictyon constrictum* (Gregory) D. G. Mann in Round *et al.*, *Tryblionella hungarica* (Grunow) D. G. Mann in Round *et al.* were dominant (relative abundance reached 5% in at least one sample) in those ponds, where *G. jadwigiae* also occurred. This is the first Hungarian occurrence of *G. jadwigiae*. The physico-chemical variables of those ponds where *G. jadwigiae* occurred are in Table 1.

Gomphonema jadwigiae Lange-Bertalot & E. Reichardt (Lange-Bertalot & Metzeltin 1996: 70, pl. 98: Figs 11–14)

Description*:* In the original description, the headpole of the species is shortly rostrate to apiculate. Our specimens are rostrate. The length of the Apaj' population is 27–40 µm, width at mid-valve is ranging from 7.6 to 9.4 µm (n = 30). Striae run parallel in mid-valve and becoming radiate towards the footpole and headpole (Figs 1–5). Number of striae is 10–14 in 10 µm. Axial area is narrow, linear (Figs 1–5). One pore present at the end of long central stria, opposite of it is a shortened stria, composed of 7 externally slit-like, internally rectangular areolae (Figs 7–11). The pore is well distinguishable from the stria, its external opening is small, round shape (Fig. 6), internally slit-like (Fig. 7). The raphe is lateral and slightly undulate with simple, slightly expanded proximal raphe endings (Figs 8–10). External proximal raphe endings are tear-drop shaped and slightly deflected towards the pore (Fig. 6). Distal raphe endings are unilaterally deflected opposite of the pore-bearing side, extending onto the valve mantle (Figs 8–9). Internally the distal raphe endings terminate in a small helictoglossa (Figs 10–11),

Table 1. The values of the environmental variables recorded for those bomb crater ponds where *G. jadwigiae* occurred.

Pond N°	**10**	**42**	**69**
Area (m^2)	28.3	28.3	38.5
Depth (cm)	50	31	44
Salinity ($g\ L^{-1}$)	2.8	2.8	2.5
Conductivity ($mS\ cm^{-1}$)	3.7	3.7	3.3
pH	8.5	8.7	8.7
Secchi-depth (cm)	27.0	9.0	10.0
Turbidity (NTU)	34	104.0	128.0
Total suspended solids ($mg\ L^{-1}$)	26.6	54.3	106.0
Total phosphorus ($\mu g\ L^{-1}$)	124.8	138.5	533.5
Chlorophyll-*a* ($\mu g\ L^{-1}$)	9.1	9.8	4.1
Nitrate nitrogen ($mg\ L^{-1}$)	0.73	0.23	0.34
Ammonium nitrogen ($mg\ L^{-1}$)	0.06	0.09	0.13
Chloride ($mg\ L^{-1}$)	553.5	354.2	362.5
Calcium ($mg\ L^{-1}$)	155.6	10.8	20.2
Open water surface (%)	97	90	95
Submerged macrophyte coverage (%)	0	0	0
Emergent macrophyte coverage (%)	3	10	5



Figs 1–11. *Gomphonema jadwigiae.* – **Figs 1–5:** Valve face in LM. **Fig. 6:** Central area with round pore. Outside view SEM. **Fig. 7:** Central area with slit-like pore. Inside view SEM. Note the unilaterally curved hook-shaped raphe endings. **Figs 8–9:** Valve outside view SEM. Note the slit-like areolae becoming c-like near the raphe (arrow). **Figs 10–11:** Valve inside view SEM. Scale bars = 10 μm (Figs 1–5), scale bars = 2 μm (Figs 7–11), scale bar = 1 μm (Fig. 6).

the proximal raphe endings are hook-shaped, curved towards the pore-bearing side (Fig. 7). The foot pole has a typical differentiated apical pore field with porelli, separated from striae by a hyaline area, and divided into two parts by the raphe (Figs 8–11). Externally the striae are composed of slit-like areolae becoming c-like near the raphe (Figs 8–9).

DISCUSSION

The studied bomb crater ponds are regarded as an excellent ecological model system (Vad *et al.* 2017) which allows us to get knowledge about the structure of the diatom communities inhabiting intermittent waters having different salinity, caused by the varying width of the macrophyte belt surrounding the ponds (Földi *et al.* 2018). The pond-system maintains high beta-diversity, which has nature conservation importance (Vad *et al.* 2017).

The species of the genus *Gomphonema* are relatively common in freshwater benthic communities (Wojtal 2003), but are not so typical for saline environments. For example in the reviews of Central European soda pans, the authors reported on the presence of only a few *Gomphonema* species (Stenger-Kovács & Lengyel 2015, Stenger-Kovács *et al.* 2018). However, as we mentioned earlier, diatom communities of these bomb crater ponds showed high similarity to those of natural astatic soda pans belonging to the biological type 3 according to the Hungarian typology (Földi *et al.* 2018, Vad *et al.* 2017), but *Gomphonema jadwigiae* has not been found yet in these pans. The species resembles *Gomphonema angustatum* (Kütz.) Rabenhorst, which was also dominant in our samples, but the structure of areolae and the internal view of pore clearly distinguish them in SEM. *G. angustatum* has c-like areolae and internally the pore is round, while *G. jadwigiae* has slit-like areolae becoming c-like near the raphe and internally the pore is slit-like. Furthermore, *G. jadwigiae* resembles *Gomphonema latelanceolatum* Levkov, Mitic-Kopanja & E. Reichardt as well, but the areolae of the latter are covered by siliceous flap (Levkov *et al.* 2016).

Gomphonema jadwigiae was described as an oligotrophic indicator in 1996 (Lange-Bertalot & Metzeltin 1996). Our specimens are less capitate than the type ones (see T65 Figs 20–21 and T98 Figs 11–14 in Lange-Bertalot & Metzeltin 1996). Because no raw material is available (pers. comm. of Lange-Bertalot and Reichardt) for SEM studies, in 2016 we collected samples from the type locality, but unfortunately we could not find the species in the samples. So for the correct identification of the species found in Apaj we used the micrographs published in Levkov *et al.* (2016). The only difference is the less capitate headpole of the Apaj' population, however some specimens of the Macedonian population also have no capitate end (see P81 Figs 2–5 and P82 Fig. 1 in Levkov *et al.* 2016). So we consider that our species is identical to Levkov's one published as *G. jadwigiae* (Levkov *et al.* 2016).

This is the first Hungarian occurrence of *Gomphonema jadwigiae*. We found it in three hypertrophic bomb crater ponds (the total phosphorus concentration in these ponds was above 100 μg L^{-1}, which is the limit of hypertrophic conditions according to the OECD 1982). *G. jadwigiae* is a worldwide rare species. It was noted as an 'interesting record' in the monitoring of benthic diatoms of lakes in Brandenburg (Werner 2014). Moreover, it has been found in the Nielba River (Poland) (Messyasz *et al.* 2011), in the eutrophic Lake Dojran and the Vardar River, Macedonia (Levkov *et al.* 2016), in a meso-eutrophic–eutrophic pond (Orangery lake) of the Botanical Garden Meise (Belgium) (Foets 2016), in a mesotrophic lake in Russia (Lake Glubokoye near Moskow) (Csudajev & Gololobova 2016), in the eutrophic Lake Villadangos (southeast León, northwest Spain) (Blanco *et al.* 2017), and in two small rivers of Hainan Island (China) (Zhi-Xin *et al.* 2018). Except in Nielba River, Lake Villadangos, and our population, the species was not dominant in the samples. Our findings broaden and clarify our knowledge of the indications as well. Because *Gomphonema jadwigiae* recently occurred mainly in eutrophic habitats, we do not think that it is an oligotrophic indicator species as it was regarded in the first description. The species can tolerate the elevated salinity and nutrient concentration.

CONCLUSIONS

Extreme habitats, like small, sodic bomb crater ponds, even if they are artificial ones, may host rarely occurring biota and contribute to the maintenance of biodiversity. This finding is an important message for habitat management and nature conservation. Moreover, our findings broaden and clarify our knowledge of the indications. On the basis of our results we do not think that *Gomphonema jadwigiae* is an oligotrophic indicator as it was regarded in the first description, because this species can tolerate the elevated salinity and nutrient concentration as well. Our results confirm the benefit of SEM for verifying the identity of diatoms, even if it is relatively of large-size, especially for routine monitoring allowing more precise ecological assessment.

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Összefoglaló: A *Gomphonema jadwigiae* Lange-Bertalot & E. Reichardt fajt az ausztriai Lermoos-hoz közeli tóból írták le 1996-ban, fénymikroszkópos morfológiai bélyegek alapján, a fajról csak ilyen képeket közölve. Ezt a kovaalgát a Kiskunsági Nemzeti Park területén, Apaj község határában található bombatölcsérekben kialakult hipertróf (az OECD 1982 által kidolgozott 100 $\mu g\ L^{-1}$ összes foszfor határérték feletti) szikes tavakban találtuk meg (egyikben dominánsként is), mely a faj eddig ismert egyetlen hazai előfordulási helye, de világszerte is kevés helyről került eddig elő. A rendelkezésre álló határozókönyvek és irodalom alapján a fajt *G. jadwigiae*-ként azonosítottuk. Ugyanakkor feltűnő volt, hogy az apaji populáció kevésbé fejecskés kovahéjjal rendelkezik, mint a típuspopuláció, azonban Lekov *et al.* (2016) határozókönyvében szintén láthatóak az apaji populációhoz hasonlóan kevéssé fejecskés példányok az ott közölt macedón populációban. Mivel típusanyag nem állt rendelkezésre, mintát vettünk a faj típuslelőhelyéről. Azonban a mintából sajnos nem került elő a *G. jadwigiae*, így a Levkov *et al.* (2016) határozókönyvében leírtakra tudtunk csak támaszkodni; az ott közölt finomszerkezeti leírással, fény- és elektronmikroszkópos képekkel hasonlítottuk össze a populációnkat, melyet szintén fény- és elektronmikroszkóppal is vizsgáltunk. Habár fénymikroszkópban emlékeztet a *Gomphonema angustatum*-ra (amelyik szintén domináns volt a vizsgált bombatölcsérekben) és a *Gomphonema latelanceolatum*-ra, de elektronmikroszkóppal egyértelműen meg lehet különböztetni tőlük, elsősorban az areola-szerkezet alapján. Ez egyben felhívja a figyelmet az elektronmikroszkóp használatának a fontosságára még ilyen relatíve nagyméretű faj esetében is, hiszen számos esetben az azonosítás csak a finomszerkezet alapján tehető meg megbízhatóan. A pontos taxonómiai identifikációnak nagy jelentősége van az ökológiai állapotértékelésben is, különösen, ha egy faj domináns a mintában.

Az általunk vizsgált populáció jó egyezést mutat a Levkov *et al.* (2016) által publikált *G. jadwigiae*-val. A fajt eredetileg oligotróf indikátorként írták le, azonban sem a többi előfordulási adata, sem az apaji populáció élőhelyének kémiai jellemzői nem támasztják alá ezt az indikációs tulajdonságot. Eredményeink alapján úgy gondoljuk, hogy a faj képes tolerálni az emelkedettebb só- és tápanyag-koncentrációt is.

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