Nitzschia austriaca Hustedt: a characteristic diatom of Hungarian inland saline waters including a morphological comparison with the type material

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Abstract

A detailed scanning electron microscopic investigation was carried out to clarify the taxonomic status of a small sigmoid *Nitzschia* species, a potential indicator of Central European soda waters. We found this taxon as one of the dominant epiphytic diatom in samples collected from sodic bomb crater ponds at Apaj (Hungary). The large population allowed for a morphometric comparison based on frustule ultrastructure with the type material of the most similar species, *Nitzschia austriaca* Hustedt that was originally described from a soda pan in the region. The results clearly demonstrated an overlap between the Apaj population and the type material of *N. austriaca* (based on NMDS analysis), therefore we argue that they represent the same taxon. An emended diagnosis of *N. austriaca* is given. To identify the main environmental variables predicting the occurrence of the species, total suspended solids and total phosphorous proved to be the most important factors, with possible interactive effects of conductivity and pH. We then expanded the distribution of the species by revisiting data originating from previous large-scale surveys targeting sodic habitats in Hungary. On the basis of our results, *N. austriaca* is a characteristic species for Central European soda waters, including the protected astatic soda pans, indicating their typical chemical and physical characteristics.

Key words: *Nitzschia austriaca, N. frustulum,* soda pans, sodic ponds, type material

Introduction

Diatoms are used as indicators of organic pollution and high nutrient loads (Smol & Stoermer 2010) and are greatly influenced by water salinity (Potapova 2011). Salinity has often been considered as one of the most important factors determining diatom distributions both in inland saline waters (Stenger-Kovács *et al.* 2016) and in estuaries (Trobajo *et al.* 2011). Species of the genus *Nitzschia* Hassall are cosmopolitan and can be found in almost every benthic assemblage from fresh to brackish waters (Denys & Lange-Bertalot 1998). Many of them have proved to be important indicators of environmental conditions. Therefore their correct identification is crucial for both conservation management (Lengyel *et al.* 2016) and paleolimnology (Yang *et al.* 2003, Korponai *et al.* 2010).

Soda waters are inland saline habitats dominated by Na^+, CO_3^- and HCO_3^- ions (Hammer 1986). In Europe, they are restricted to the lowland parts of the Carpathian basin (Boros *et al.* 2014). Among them, soda pans are shallow, astatic water bodies with a relatively large areal extent. They are inhabited by several rare and halophilic species, and attract diverse and abundant waterfowl communities (Boros *et al.* 2013, Horváth *et al.* 2013). With the implementation of the European Water Framework Directive and recognition of the conservation values of the astatic soda pans, a number of studies of their benthic diatoms have recently been started (Stenger-Kovács *et al.* 2014a,b, 2016, Stenger-Kovács & Lengyel 2015, Lengyel *et al.* 2016).

One of the most common (sometimes dominant), sigmoid *Nitzschia* species has not yet been identified to species level; in a recently published taxonomic guide of diatoms of Central European soda pans (Stenger-Kovács & Lengyel 2015), it is identified as *Nitzschia* sp. 1. Given it is a widespread and often dominant member of diatom communities of these inland saline waters, its precise identification is a crucial step for ecological analyses (Morales *et al.* 2001).

We found this *Nitzschia* species as a dominant epiphytic diatom in several sodic bomb crater ponds at Apajpuszta (Apaj plain), Central Hungary (indicated as *Nitzschia* sp. 2 in Vad *et al.* 2017), allowing us the opportunity to perform a detailed electron microscopical investigation.

The main objectives of this study were 1) to compare this dominant *Nitzschia* species with other similar *Nitzschia* species based on light microscopy (LM) observations; 2) to provide a detailed morphological description of it using scanning electron microscope (SEM) information. Further, incorporating an ultrastructural study of the type material of the most similar species, *Nitzschia austriaca* Hustedt (1959: 439, figs 28–31), also illustrated by Simonsen (1987: 458–459, pl. 685, figs 15–22, holotype figs 15–16); 3) to identify the main benthic diatom taxa present in bomb crater ponds; 4) provide details on the ecological demands of *N. austriaca* based on its occurrence in the sodic ponds.

Material and methods

A dense cluster of sodic bomb crater ponds (N=112) is situated in the northernmost part of the Kiskunság National Park (47°7.403' N 19°8.187' E), near to the village of Apaj. The ponds are on the plain of Danube-Tisa Interfluve, Central Hungary in an area of approximately 25 hectares. Their salinity ranges from freshwater to moderately saline levels (with conductivities from 1.3 to 7.1 mS cm^{-1}). More information of the study sites is available in Vad *et al.* (2017).

Altogether 48 ponds were sampled for diatoms. Benthic diatom samples were taken from 10 cm sections (or maximal length if the depth of pond was less than 10 cm) of green stems of common reed (*Phragmites australis* (Cav.) Trin. ex Steud), 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. At the same time, the following physico-chemical variables were measured: water depth, pond's diameter, 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^-). The determination methods and more details 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 light microscope (LM) observations. At least 400 valves were counted in each preparation to estimate the relative abundance of each taxon in the sample. For scanning electron microscope (SEM) studies a part of the cleaned and washed samples was filtered through a 3 µm Isopore™ polycarbonate membrane filter (Merck Millipore), that can retain the smallest diatom valves, but filter undesirable inorganic particles. The membrane filter 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 15 kV and 10 mm distance using secondary electrons (SE) and scanning transmission electron microscopy (STEM) detectors.

The type material of *Nitzschia austriaca* Hustedt (sample E9708, “Burgenland, Österreich Ober Schrändl”) from the Friedrich Hustedt Collection (Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven) was also investigated with SEM.

Statistical analysis

The valve length (L), width (W), number of striae (S) and fibulae (F) in 10 µm were measured and counted on SEM micrographs (74 valves of the Apaj population and 22 valves of the type material). The normality of data was tested by Shapiro-Wilk test. Levene’s tests based on medians were carried out to examine the homogeneity of variances. As the data deviated significantly from normal distribution, a Mann-Whitney test was carried out to compare the medians of the metrics. Non-metric multidimensional scaling (NMDS) with Euclidean similarity index was carried out to check if there was a clear separation between the type material and the Apaj *Nitzschia* population based on the recorded morphological characters. The multivariate statistics and tests were calculated with software PAST version 1.78 (Hammer *et al.* 2001).

To identify the most important environmental variables influencing the presence of *N. austriaca* among the bomb crater ponds (N=48) we used a multiple logistic regression. As dependent variable, we used presence-absence data of the species. Our explanatory variable set consisted of TSS, TP, DIN (sum of NO_3-N and NH_4-N) that were ln-transformed, Chl-*a* (square-root transformed), and the ratio of open water (arcsin-transformed). Transformations were performed in order to normalise their distribution. Then we applied back and forward stepwise model selection (the mode of stepwise search was ′both′, number of permutations: 999) based on the Akaike information criterion (AIC) with the ′stepAIC′ function of the R package ′MASS′ (Venables & Ripley 2002). AIC estimates the relative quality of the models for a given set of data. The overall significance of the model with the lowest AIC value was tested with the likelihood ratio test.

Results

Morphological comparison of Nitzschia austriaca *valves from Apaj (Hungary) and type material (Austria)*

The data of length, width, the number of striae and fibulae of the population from Apaj and of the type material overlapped with each other (Table 1). A Mann-Whitney test showed that the medians of the striae and fibulae number were equal, but the width and lengths were not equal in the two studied populations. The results of NMDS also display that the metrics of both populations overlapped, which was also confirmed by the Shepard plot (Fig. 1). Based on these results, we argue that the species of the population from Apaj is the same as the type material of *Nitzschia austriaca*.

Nitzschia austriaca *based on the Apaj populations and type material*

The length, width, number of striae and fibulae in 10 μm of Apaj populations and type material are available in Table 1. The shape of valves is sigmoid with slightly capitate ends (Figs 2, 12, 20, 22). Transapical striae are visible in LM (Figs 2–13). The fibulae are quite regularly distributed along the raphe canal, with the two median ones usually further apart from each other (Figs 18, 19, 23, 24). In SEM, externally: the striae are uniseriate and areolae are circular (Figs 15, 21). On the mantle, the striae are very short, comprising one areola (Figs 14, 21). The distal raphe endings turn always to the same side, either toward the valve face (Figs 14, 20) or the valve mantle (Figs 16, 21). Central raphe fissures are drop-shaped and close to each other, the proximal raphe end follows the valve margin (Figs 15, 17, 20, 21). Internally: areolae are not covered by hymenes (Figs 19, 24). The raphe slit is interrupted by a central nodule (Figs 18, 24). The distal raphe endings end in a helictoglossa (Figs 19, 24).

Hungarian distribution of Nitzschia austriaca

This sigmoid *Nitzschia* species was reported several times during the Hungarian surveillance monitoring. Because of the uncertainity of its identification, we reinvestigated those samples where it was indicated as dominant. Based on this, we revealed that *Nitzschia austriaca* occurs in several Hungarian astatic soda pans additional to its presence in the sodic ponds of

Apaj. These pans are the followings: Nagyvadas-tó (at the settlement Császárszállás: N 47°51’37.99”; E 21°41’38.52”); Kelemen-szék (Fülöpszállás: N 46°47’36.76”; E 19°10’29.43”); Kolon-tó (Izsák: N 46°46’05.08”; E 19°19’47.78”); Nádas-tó (Sándorfalva: N 46°23’14.59”; E 20°05’44.93”); Büdös-szék (Szabadszállás: N 46°51’51.27”; E 19°10’01.75”); Zabszék (Szabadszállás: N 46°49’43.02”; E 19°10’38.88”); Böddi-szék (Dunatetétlen: N 46°46’03.64”; E 19°09’03.86”); Bába-szék (Dunatetétlen: N 46°44’23.83”; E 19°09’01.08”); Nagy-Széksóstó (Mórahalom: N 46°12’41.71”; E 19°57’07.23”); Csikóspusztai-tó (Királyhegyes: N 46°17’33.98”; E 20°38’39.12”); Pusztaszeri Büdös-szék (Pusztaszer: N 46°32’58.53”; E 20°01’45.44”); Kis Sóstó (Csongrád: N 46°44’38.83”; E 19°57’34.57”) (Fig. 25).

Dominant diatoms

Nitzschia austriaca occurred in 30 ponds among the 48 sampled, but it was prominent (relative abundance ≥ 5%) only in 12. Its relative abundance varied between 0.2 to 54.5% (Fig. 26). Moreover, eighteen species were prominent (relative abundance reached the 5% at least in one sample) in those ponds, where *N. austriaca* also occurred (Table 2, Supplementary Table 1). The relative abundance of *N. austriaca* was the highest (RA: 54.5%) in the pond N^{o} 111 with co-dominance of *Halamphora dominici* Acs & Levkov (2009: 185, pl. 90, 17–28; pl. 210, 4–7) (RA: 31.7%). This pond had the highest TSS (388 mg L^{-1}).

Distribution of N. austriaca *in relation to environmental variables*

Altogether 48 ponds were sampled, but here we give the physico-chemical variables for 30/12 ponds, where *Nitzschia austriaca* occurred or was dominant. These 30 ponds were alkali (pH 7.8–9), their trophic conditions varied from eutrophic to hypertrophic (TP 41.7–1693.6 µg L^{-1} (Table 3) according to OECD (1982). On average salinity was 2.9 g L^{-1} and electric conductance was 3.8 mS cm^{-1}.

TSS and TP were retained in the multiple logistic regression model with the lowest AIC value (χ^2 = 25.5, df = 2, p<0.001), being the most important variables predicting the occurrence of the species. TSS and TP, along with conductivity and pH were significantly higher in ponds where *N. austriaca* occurred (Fig. 27). Moreover these ponds were more shallow, turbid and larger than the others and the DIN concentration was lower (Table 3).

Discussion

The taxonomical position of *Nitzschia austriaca* has been confusing. Krammer & Lange-Bertalot (1988) questioned whether *N. austriaca* could be a synonym of *N. frustulum* (Kützing) Grunow (Kützing 1844: 63, fig. 77). Further, Aboal *et al.* (2003) forgot to put the question mark into their compilation (pers. comm. of M. Aboal) and treated *N. austriaca* as a synonym of *N. frustulum* when observed in Spanish occurrences. Aboal *et al.* (2003) is given as the key reference on Algaebase (Guiry & Guiry 2017) and other on-line portals with the following comment on its taxonomic status concerning *Nitschia austriaca*: "This name is currently regarded as a taxonomic synonym of *Nitzschia frustulum* (Kützing) Grunow". Probably several authors have accepted this species concept. For example Kociolek & Herbst (1992: 348, figs 22–24, 35, 36) named a *N. austriaca*-shaped form as *N. frustulum,* though they remark that it is a *N. austriaca*-like cell, showing robust fibulae and distinct helictoglossae in SEM (figs 35, 36). Inversely, in the appendix of the checklist of diatom species reported from Canadian coastal waters, Mather *et al.* (2010) indicated *N. austriaca* as valid name and its synonym was *N. frustulum*. Probably this is the reason why Souffreau *et al.* (2010: fig. 24) named a *N. frustulum*-like diatom as *N. austriaca.*

As this small sigmoid *Nitzschia* was very abundant and a frequent species in our samples, we started detailed morphometric and SEM investigations to clarify its taxonomic status. According to our result, the morphological and morphometric characteristics of both populations (type material and bomb crater ponds from Apaj) overlapped with each other (Fig. 1). Based on this, the populations from the bomb crater ponds and the type material are undoubtedly identical.

The presence of *Nitzschia austriaca* has been doubtful in Hungary. It was first recorded as a dominant species (without any micrographs) during the investigations of soda pans in the regions Fertő-Hanság and Danube–Tisza Interfluve (Lengyel *et al.* 2012, Stenger-Kovács 2013, Stenger-Kovács *et al.* 2014a,b). However, the recent taxonomic and distribution guide of diatom taxa of Central European soda pans does not include it (Stenger-Kovács & Lengyel 2015). They reported a very similar taxon to *N. austriaca* as *Nitzschia* sp. 1 which frequently occurred as dominant in this area. The authors provided a comment on this taxon: "Similar to *Nitzschia austriaca*, however, *N. austriaca* is thinner (width: 1.8–2.4 µm)" (Stenger-Kovács & Lengyel 2015). According to the morphological variability of the valve width found in our study, we suggest that *Nitzschia* sp. 1 reported by these authors most probably belongs to *N. austriaca*.

According to Hustedt's description, the cells of *N. austriaca* in girdle view are narrow linear and in valvar view sigmoid. The cells are 12–22 µm long and 1.8–2.4 µm width. The ends of cells are slightly capitate. Fibulae are eccentric, 12 in 10 µm. Striation is visible, transapical striae are about 22 in 10 µm. In the type material of *N. austriaca* we found minimally 14, maximally 23.4 µm long, and minimally 2.3, maximally 2.8 µm wide specimens (based on SEM micrographs). The stria density varied between 26 and 29 in 10 µm and the fibula density was 12–15 in 10 µm. The differences were probably caused either by the differences in the applied measuring methods (we measured these metrics from the SEM micrographs, which is a more precise method than light microscopic measuring), or the differences in the number of measured specimens in our study and during the original description. The range of metrics of the Apaj population is wider than that of the Austrian type material which is likely due by the lower number of measured valves from the type material. Another possible explanation of the difference is the effect of salinity. Trobajo *et al.* (2011) demonstrated that the length (L), width (W) of valves and L:W ration of *Nitzschia* species could change under different salinity conditions.

Nitzschia austriaca clearly differs from *N. frustulum* in shape. According to Trobajo *et al.* (2013) who reinvestigated the type materials of five problematic *Nitzschia* taxa providing clear criteria for their identification, the shape of *N. frustulum* valves are linear to lanceolate, while *N. austriaca* are sigmoid (Hustedt 1959). *Nitzschia austriaca* also differs from the other sigmoid *Nitzschia* species: *Nitzschia aremonica* R.E.M. Archibald (1983: 237–238, figs 359–360), *N. kurzeana* Rabenhorst (1873: no. 2312), *N. obtusa* W. Smith (1853: 39, pl. 13, fig. 109) and *N. sigma* (Kützing 1844: 67, pl. 30, fig. 14) W. Smith (1853: 39, pl. 13, fig. 108) are all significantly longer and wider than *N. austriaca*. *Nitzschia nana* Grunow in Van Heurck (1881: pl. 67, fig. 3) is significantly longer and has denser striation, while *N. brevissima* Grunow in Van Heurck (1881: pl. 67, fig. 4) is wider with denser striation in contrast with *N. austriaca.* The proximal raphe end of *N. clausii* Hantzsch (1860: 40, pl. 6, fig. 7) (fig. 2a' in Bes & Torgan 2010, fig. 4b in Sala *et al.* 2015), *N. filiformis* var. *conferta* (P.G. Richter 1879: 65) Lange-Bertalot in Lange-Bertalot & Krammer (1987: 18) (fig. 4n in Sala *et al.* 2015) and *N. scalpelliformis* Grunow in Cleve & Grunow (1880: 92) (Syn: *N. parvuloides* Cholnoky (1955: 179, figs 72–73) – pl. 1, fig. 5 in Lange-Bertalot & Krammer 1987) curves away from the margin toward the valve center, while that of *N. austriaca* follows the valve margin.

Nitzschia terrestris (J.B. Petersen 1928: 418, fig. 31) Hustedt (1934: 396) has significantly less fibulae and more striae unlike *N. austriaca* (Supplementary Table 2).

The existing records suggest that *N. austriaca* is a specialist of saline waters, although the correct identification of this species is questionable in some cases where no picture was shown (Supplementary Table 3). According to our results, the presence of *N. austriaca* was mostly driven by TSS and TP. High TSS and TP coupled with high pH are typical features of the inland saline waters, soda pans (Boros *et al.* 2017). In soda pan habitats, these variables are correlating with each other (Horváth *et al.* 2014), such as in the sodic bomb crater ponds of the present study (Vad *et al.* 2017) and therefore, it is hard to separate their unique effects on the species. In line with Hustedt (1959), who regarded *N. austriaca* as a characteristic species of soda lakes, we suggest, that *N. austriaca* can be used as a reliable indicator species of soda waters, including the protected astatic soda pans.

Nitzschia austriaca Hustedt (**emend.** Ács & Ector)

Valve length 12–34 µm, width 1.8–3.3 µm, 22–30 striae in 10 µm, 12–16 fibulae in 10 µm. In SEM, externally: the striae are uniseriate and are never doubled on the raphe keel. Areolae are circular. On the mantle, the striae are very short, comprising one areola. The distal raphe endings turn always to the same side, either toward the valve face or the valve mantle. Central raphe fissures are drop-shaped and close to each other, the proximal raphe end follows the valve margin. Internally: areolae are not covered by hymenes. The raphe slit is interrupted by a central nodule. A small helictoglossa is present internally at both distal raphe endings.

The species is frequent in Hungarian soda pans and likely widespread in other inland saline lakes, but due to the uncertainity of its taxonomical position it has probably been identified sometimes as *Nitzschia frustulum*, despite the distinct sigmoid outline of the valves. *Nitzschia austriaca* prefers turbid, hypertrophic soda waters.

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Table and Figure captions

TABLE 1. Morphometric analysis of *Nitzschia austriaca* in Apaj (A) and type material (T). Abbreviations: L: length, W: width, S: number of striae in 10 μm, F: number of fibulae in 10 μm.

TABLE 2. List of the dominant taxa with their maximum relative abundance (RA) in those ponds where *N. austriaca* occurred.

TABLE 3. Ranges, mean and median values of the environmental variables recorded in May 2014 for those bomb crater ponds. Mean and median values and errors (occurred/dominant) for *N. austriaca*.

SUPPLEMENTARY TABLE 1. The relative abundance of dominant species in those ponds where *N. austriaca* occurred. See abbreviation in Table 2.

SUPPLEMENTARY TABLE 2. Light microscopic morphological features of *Nitzschia austriaca* and some similar sigmoid *Nitzschia*.

SUPPLEMENTARY TABLE 3 Worldwide citations of *Nitzschia austriaca*, where the authors did not show any pictures.

FIGURE 1. Ordination diagram of NMDS (a) with the 95% confidence interval (point: population of Apaj, plus: population of type material) and the shepard plot (b). R^2=0.95.

FIGURES 2–13. LM micrographs of *N. austriaca* from the Apaj population (Hungary).

FIGURES 14–19. SEM micrographs of *N. austriaca* from the Apaj population (Hungary). Arrow indicates the central raphe fissures on Fig. 15 and the central nodule on Fig. 18.

FIGURES 20–24. SEM micrographs of *N. austriaca* from the type material (sample E9708 from Austria). Arrow indicates the central nodule on Fig. 24.

FIGURE 25. The Hungarian occurrences of *N. austriaca*. Dot: Hungarian surveillance monitoring and our former data, star: data from present study.

FIGURE 26. Relative abundance of *N. austriaca* (NAUS) in the selected ponds where it occurred.

FIGURE 27. Box plots showing variation in chemical variables, (a) conductivity, (b) pH, (c) TSS and (d) TP values of ponds, where *N. austriaca* is present (1) or absent (0).

TABLE 1.

	A_L (µm)	T_L (µm)	A_W (µm)	T_W (µm)	A_S/10µm	T_S/10µm	A_F/10µm	T_F/10µm
Min	13.6	14	2.4	2.3	26	26	12	12
Max	34.0	23.4	3.3	2.8	30	29	16	15
Mean±SE	20.4±0.5	17.3±0.6	2.7±0.02	2.5±0.03	27.6±0.11	27.5±0.21	13.6±0.17	13.7±0.26
Median	18.8	16.9	2.7	2.5	28	27.5	13	14

TABLE 2.

	code	**maximal RA (%)**
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI	20,6
Craticula buderi (Hustedt) Lange-Bertalot	CRBU	10,1
Eunotia bilunaris (Ehrenberg) Mills var. *bilunaris*	EBIL	5,9
Gomphonema angustatum (Kützing) Rabenhorst	GANG	50,8
Gomphonema cf. *micropus* Kützing	GMIC	14,1
Gomphonema clavatum Ehrenberg	GCLA	15,3
Gomphonema jadwigiae Lange-Bertalot & E. Reichardt	GJAD	8,9
Halamphora dominici Ács & Levkov	HDOM	51,0
Halamphora paraveneta (Lange-Bertalot, Cavacini, Tagliaventi & Alfinito) Levkov	HPVE	13,0
Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS	6,1
Navicula veneta Kützing	NVEN	67,2
Navicula wiesneri Lange-Bertalot	NWIE	16,9
Nitzschia frustulum (Kützing) Grunow var. *frustulum*	NIFR	49,8
Nitzschia liebetruthii Rabenhorst var. *liebetruthii*	NLBT	30,9
Nitzschia pusilla (Kützing) Grunow (emend Lange-Bertalot)	NIPU	12,6
Nitzschia austriaca Hustedt	NAUS	54,5
Nitzschia supralitorea Lange-Bertalot	NZSU	21,7
Psammodictyon constrictum (W. Gregory) D.G. Mann	PCON	25,2
Tryblionella hungarica (Grunow) D.G. Mann	THUN	5,2

TABLE 3.

	Min	**Max**	**Mean ± SE**	**Median**
Area (m^2)	7.1/12.6	86.5	42.4 ± 3.9 / 48.6 ± 7.1	38.5 / 44.4
Depth (cm)	4	60 / 45	34.9 ± 2.5 / 28.5 ± 3.9	34.4 / 33
Salinity (g L^{-1})	1.4	4	2.9 ± 0.1/ 3 ± 0.2	3 / 3.3
Conductivity (mS cm^{-1})	1.8	5.1	3.8 ± 0.2 / 3.9 ± 0.3	3.8 / 4.2
pH	7.8/8	9	8.5 ± 0.1/8.7 ± 0.1	8.7
Secchi-depth (cm)	4	27 / 25	13.8 ± 1.3 / 10.8 ± 2.1	14 / 9.5
Turbidity (NTU)	6.1/51	691	166 ± 34.3 / 238.3 ± 62.2	81 / 175
Total suspended solids (mg L^{-1})	26.6/35.4	388	104.7 ± 18.4 / 135.9 ± 35.4	64.3 / 108
Total phosphorus (µg L^{-1})	41.7/126.9	1693.6	386 ± 65.9 / 612.6 ± 139.1	243.6 / 616.2

Chlorophyll-*a* (µg L^{-1})	0.0/4.1	388	37.7 ± 14.4 / 56.3 ± 36.8	10.4 / 12.4
Nitrate nitrogen (mg L^{-1})	0.10	0.70 / 0.47	0.30 ± 0.03 / 0.26 ± 0.03	0.30 / 0.23
Ammonium nitrogen (mg L^{-1})	0.01/0.03	3.51 / 1.01	0.41 ± 0.10 / 0.32 ± 0.09	0.15 / 0.19
Chloride (mg L^{-1})	219	1117 / 1049	554 ± 49 / 510 ± 87	520 / 421
Calcium (mg L^{-1})	8.80	156 / 67.3	45.5 ± 5.9 / 28.4 ± 5.7	39.7 / 22.8
Open water surface (%)	50.0	99.5	88.5 ± 2.2 / 87.9 ± 4.6	95.0 / 94.5
Submerged macrophyte coverage (%)	0.0	20.0	1.3 ± 0.8 / 2 ± 1.9	0.0
Emergent macrophyte coverage (%)	0.5/1	50.0	9.9 ± 2.2 / 9.3 ± 4.5	5.0

SUPPLEMENTARY TABLE 3.

Continent	**Country**	**References**
Asia	Turkey	Kivrak & Uygun 2012
Europa	Austria	Höfler & Fetzmann 1959, Hustedt 1959, Simonsen 1987, Krammer & Lange-Bertalot 1988, Yoshitake & Fukushima 1992
Europa	Hungary	Stenger-Kovács 2013, Stenger-Kovács *et al.* 2014a, b
Europa	Romania	Nagy *et al.* 2006, Cărăuş 2012
Europa	Spain	Aboal *et al.* 1996
North America	Canada	Mather *et al.* 2010
North America	USA	Bahls 2009
Oceania	Australia	Chessman *et al.* 2007
South America	Argentina	Hassan *et al.* 2011, González Achem *et al.* 2014

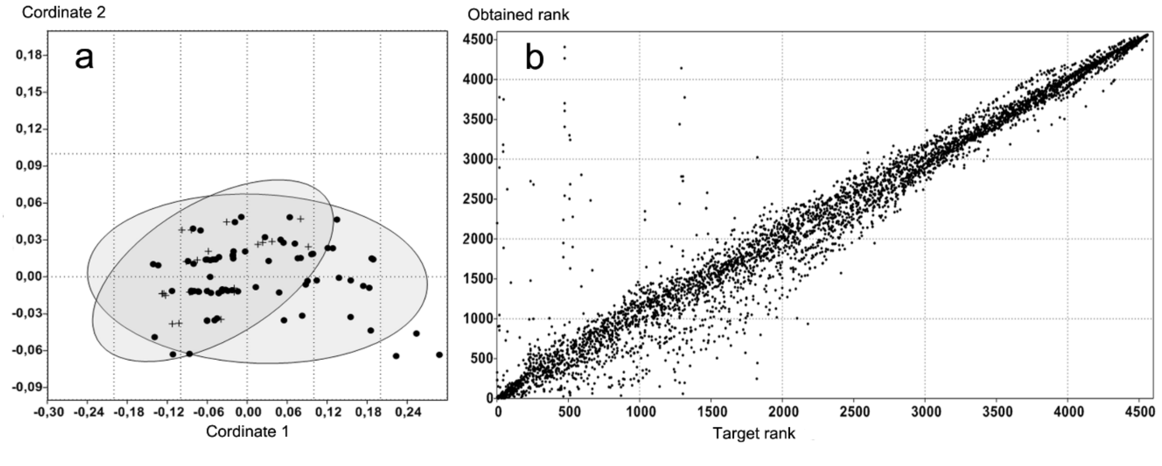
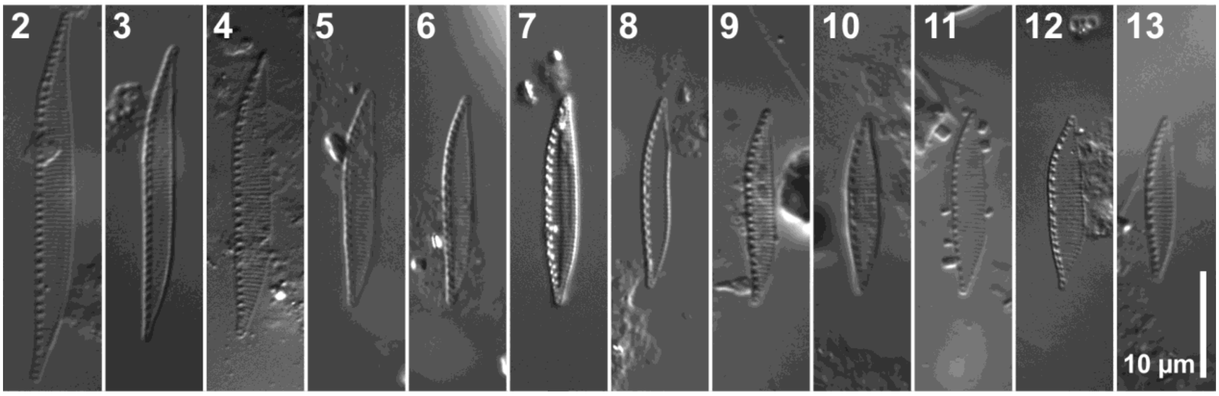
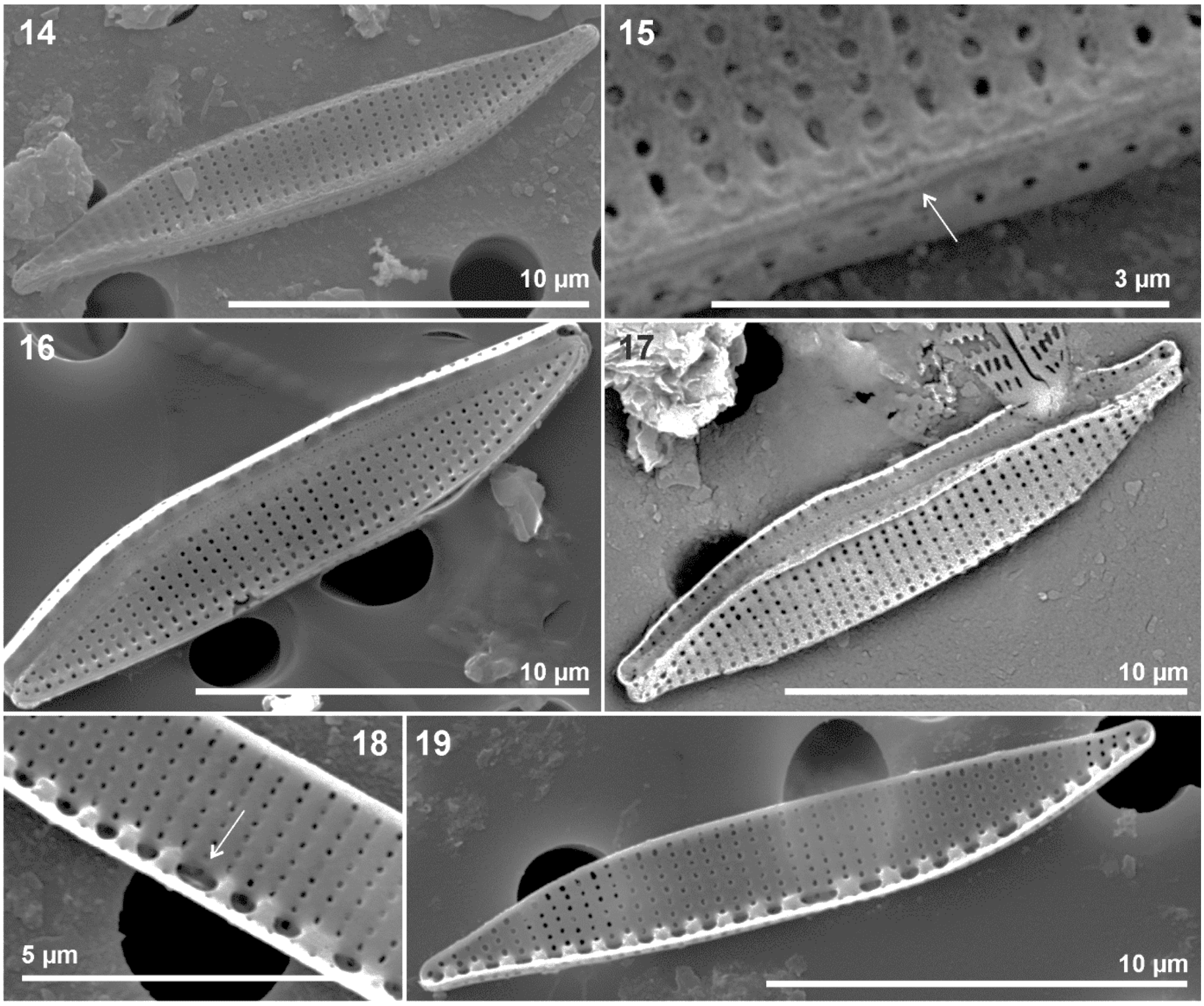


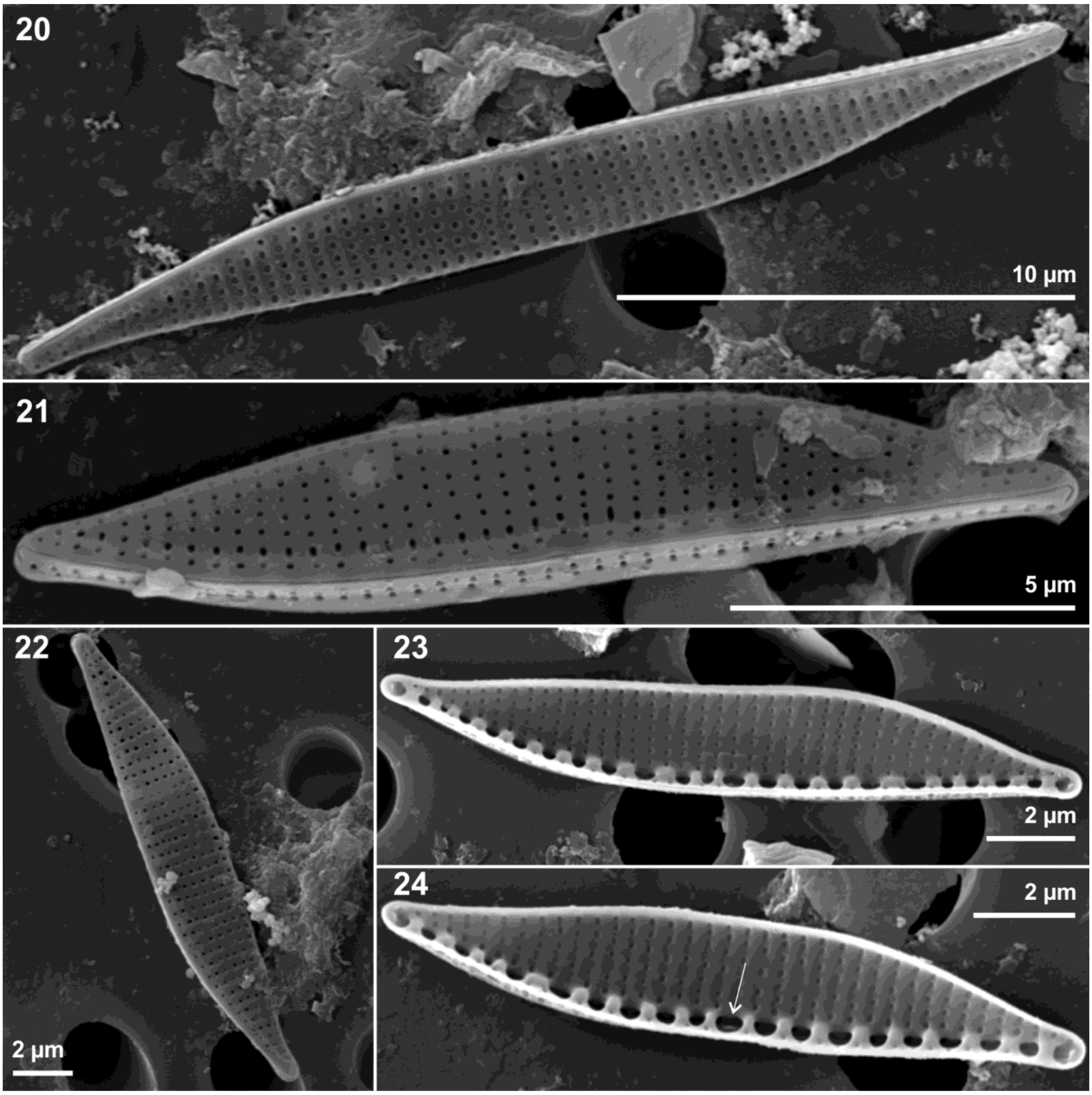
FIGURE 1.



FIGURES 2–13.



FIGURES 14–19.



FIGURES 20–24.

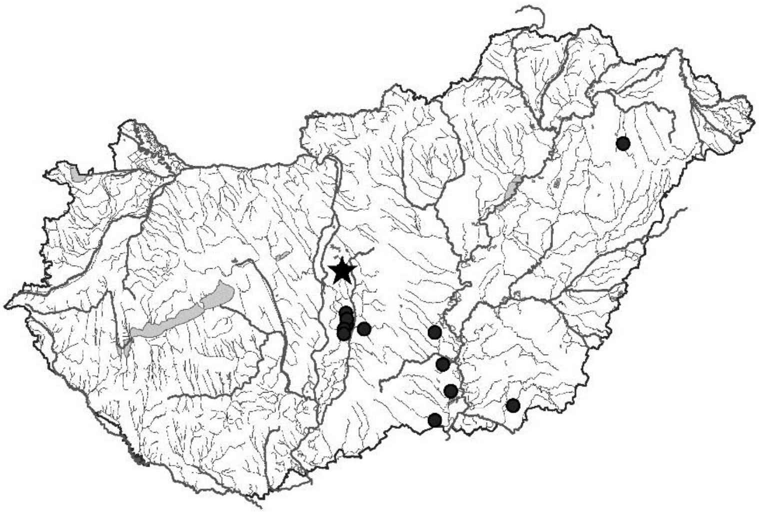


FIGURE 25.

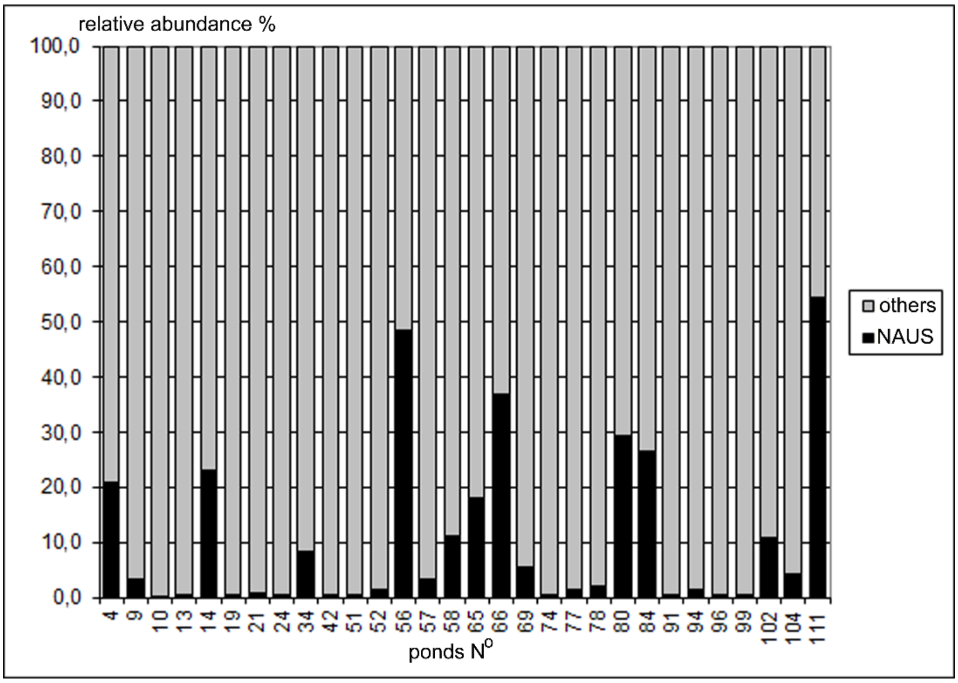


FIGURE 26.

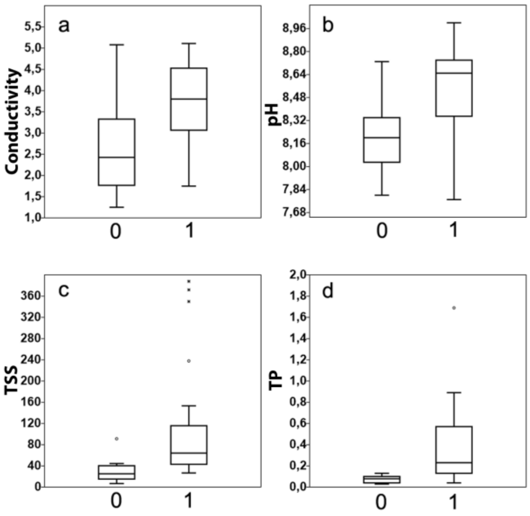

a
Conductivity
5,5
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0
1
b
pH
8,96
8,80
8,64
8,48
8,32
8,16
8,00
7,84
7,68
0
1
c
TSS
360
320
280
240
200
160
120
80
40
0
0
1
d
TP
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
1

FIGURE 27.

Supplementary Table 1. Species code abbreviation following OMNIDIA software.

Nº of pond	**ADMI**	**CRBU**	**EBIL**	**GANG**	**GMIC**	**GCLA**	**GJAD**	**HDOM**	**HPVE**	**NRCS**	**NVEN**	**NWIE**	**NIFR**	**NLBT**	**NIPU**	**NAUS**	**NZSU**	**PCON**	**THUN**
4	0,0	0,0	0,0	0,2	0,0	0,0	0,0	30,6	1,6	0,0	18,1	6,8	1,9	15,5	0,0	20,9	0,0	0,0	0,0
9	20,6	10,1	0,0	17,1	13,2	8,8	0,0	1,8	0,0	0,0	17,5	0,0	1,3	1,3	0,4	3,5	0,0	0,0	0,0
10	0,0	0,0	0,0	8,0	1,4	0,0	0,2	3,5	0,0	0,0	16,6	0,9	21,5	30,9	0,0	0,2	0,0	0,7	5,2
13	0,0	0,5	0,0	0,0	0,0	0,0	0,0	12,0	0,0	0,2	23,3	0,7	49,8	6,4	2,2	0,5	2,7	1,2	0,0
14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	18,3	2,6	0,5	33,3	2,1	4,2	8,9	0,0	23,2	4,2	2,1	0,2
19	0,0	0,0	0,0	50,8	1,4	15,3	0,0	0,2	0,5	0,0	21,0	0,0	2,4	1,0	0,2	0,5	0,2	1,2	0,0
21	0,0	0,0	0,0	0,9	0,0	0,0	0,0	7,3	0,0	0,7	50,9	0,0	4,0	5,7	0,0	0,9	0,0	19,3	4,5
24	0,0	0,0	0,0	0,5	0,0	0,0	0,0	28,8	0,0	0,0	35,2	4,3	13,0	16,5	0,0	0,7	0,0	0,2	0,0
34	0,5	3,5	5,9	8,3	0,5	1,4	0,0	5,2	0,0	4,2	23,6	0,0	12,0	10,6	0,0	8,3	0,0	2,1	0,0
42	0,0	0,7	0,0	3,3	0,0	1,9	0,7	4,9	0,0	0,7	49,3	16,0	3,0	3,3	0,0	0,7	2,3	9,5	0,0
51	0,0	2,3	0,0	50,4	10,3	0,9	0,0	0,2	0,0	1,2	16,2	0,0	7,0	7,7	0,0	0,5	0,0	0,0	0,0
52	0,0	1,2	0,0	0,0	0,0	0,0	0,0	21,1	0,2	0,0	18,5	2,1	29,7	15,2	0,0	1,4	8,4	1,4	0,2
56	0,0	0,0	0,0	0,2	0,0	0,0	0,0	16,2	0,5	0,2	19,4	8,7	0,0	2,2	0,0	48,4	2,9	0,0	0,0
57	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,2	0,0	0,2	32,1	5,7	29,9	6,4	0,0	3,3	8,1	0,5	5,2
58	0,0	0,0	0,0	0,0	0,0	0,0	0,0	51,0	13,0	0,0	5,6	1,8	0,2	0,0	0,0	11,2	16,6	0,0	0,0
65	0,0	0,0	0,0	0,0	0,0	0,0	0,0	36,3	0,0	0,0	11,1	0,5	5,9	1,4	12,6	18,0	9,2	0,0	0,0
66	0,0	0,0	0,0	0,0	0,0	0,0	0,0	28,9	0,0	0,0	8,9	16,9	4,2	0,0	0,0	36,9	3,1	0,0	0,0
69	0,0	0,0	0,0	1,4	5,5	2,8	8,9	13,3	0,7	0,0	47,0	3,4	1,1	6,0	0,0	5,5	0,9	1,1	0,0
74	0,0	0,0	0,0	0,7	14,1	0,0	0,0	10,8	0,5	0,5	41,9	4,0	15,5	8,5	0,0	0,7	0,0	2,8	0,0
77	0,0	0,0	0,0	22,6	5,9	0,0	0,0	1,6	0,0	6,1	30,4	0,5	5,2	8,9	0,0	1,4	0,0	13,9	1,9
78	0,0	0,0	0,0	2,4	0,0	0,2	0,0	16,8	0,5	1,2	48,7	3,8	19,4	1,4	0,0	2,1	1,4	0,9	0,0
80	0,0	0,0	0,0	0,0	0,0	0,0	0,0	35,4	0,0	0,0	6,5	4,9	2,1	0,0	0,0	29,4	21,7	0,0	0,0
84	0,0	0,0	0,0	0,0	0,0	0,0	0,0	46,8	1,4	0,0	16,9	0,9	3,3	0,0	0,7	26,5	3,5	0,0	0,0
91	0,0	2,1	0,0	5,4	2,6	0,0	0,0	11,3	4,7	0,0	47,6	4,0	1,2	5,2	0,0	0,5	0,5	12,5	0,0
94	0,0	0,5	0,0	2,9	3,4	0,0	0,0	10,2	0,0	0,0	49,5	11,2	0,5	2,4	0,0	1,5	15,0	1,5	0,5

96	0,0	0,2	0,0	12,0	2,8	0,0	0,0	0,9	0,0	0,9	46,0	2,1	4,0	0,9	0,0	0,5	0,2	25,2	2,4
99	0,9	0,2	0,0	12,1	3,6	0,0	0,0	8,1	0,0	0,2	29,3	4,3	18,6	5,1	0,0	0,7	3,1	7,6	2,2
102	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,6	0,0	0,0	67,2	5,9	1,1	0,0	0,0	11,0	0,0	0,0	0,0
104	0,0	0,0	0,0	0,0	0,0	0,0	0,0	47,5	0,0	0,0	44,2	0,7	1,0	0,2	0,0	4,3	1,4	0,0	0,0
111	0,0	0,0	0,0	0,0	0,0	0,0	0,0	31,7	0,0	0,0	3,5	0,5	8,9	0,0	0,0	54,5	0,0	0,0	0,0

SUPPLEMENTARY TABLE 2.

	Length (µm)	**Width (µm)**	**Striae /10 µm**	**Fibulae /10 µm**	**Central nodule**	**Ends of raphe**
Nitzschia aremonica R.E.M. Archibald (1983: 237–238, figs 359–360)	(31)38–75	4–5	24–28	7–10	no data	no data
Nitzschia austriaca Hustedt (1959: 439, figs 28–31)	12–32	1.8–3.1	25–30	12–16	Yes	proximal raphe end follows the valve margin
Nitzschia brevissima Grunow in Van Heurck (1881: pl. 67, fig. 4)	18–54	3.5–6.5	30–38	5–10	Yes	no data
Nitzschia clausii Hantzsch (1860: 40, pl. 6, fig. 7)	20–55	3–5	38–42	10–13	Yes	proximal raphe end curves away from the margin toward the valve centre
Nitzschia filiformis var. *conferta* (P.G. Richter 1879: 65) Lange-Bertalot in Lange-Bertalot & Krammer (1987: 18)	20–45	3–5	NA	NA	Yes	proximal raphe end curves away from the margin toward the valve centre
Nitzschia kurzeana Rabenhorst (1873: no. 2312)	120–350	7–13	22–30	5–6	Yes	terminal raphe fissures are distinctly recurved
Nitzschia nana Grunow in Van Heurck (1881: pl. 67, fig. 3)	35–120	3–4.5	30–36	7–11	Yes	no data
Nitzschia obtusa W. Smith (1853: 39, pl. 13, fig. 109)	55–150	7–9	28–30	6–9	Yes	no data
Nitzschia scalpelliformis Grunow in Cleve & Grunow (1880: 92)	20–110	4.5–7.4	(25)27–38	7–10	Yes	proximal raphe end curves away from the margin toward the valve centre
Nitzschia sigma (Kützing 1844: 67, pl. 30, fig. 14) W. Smith	30–200	4.0–13.0	19–24	7–12	No	proximal raphe end follows the valve margin

(1853: 39, pl. 13, fig. 108)						
Nitzschia terrestris (J.B. Petersen 1928: 418, fig. 31) Hustedt (1934: 396)	25–115	3–5	32–35	5–8	Yes	no data