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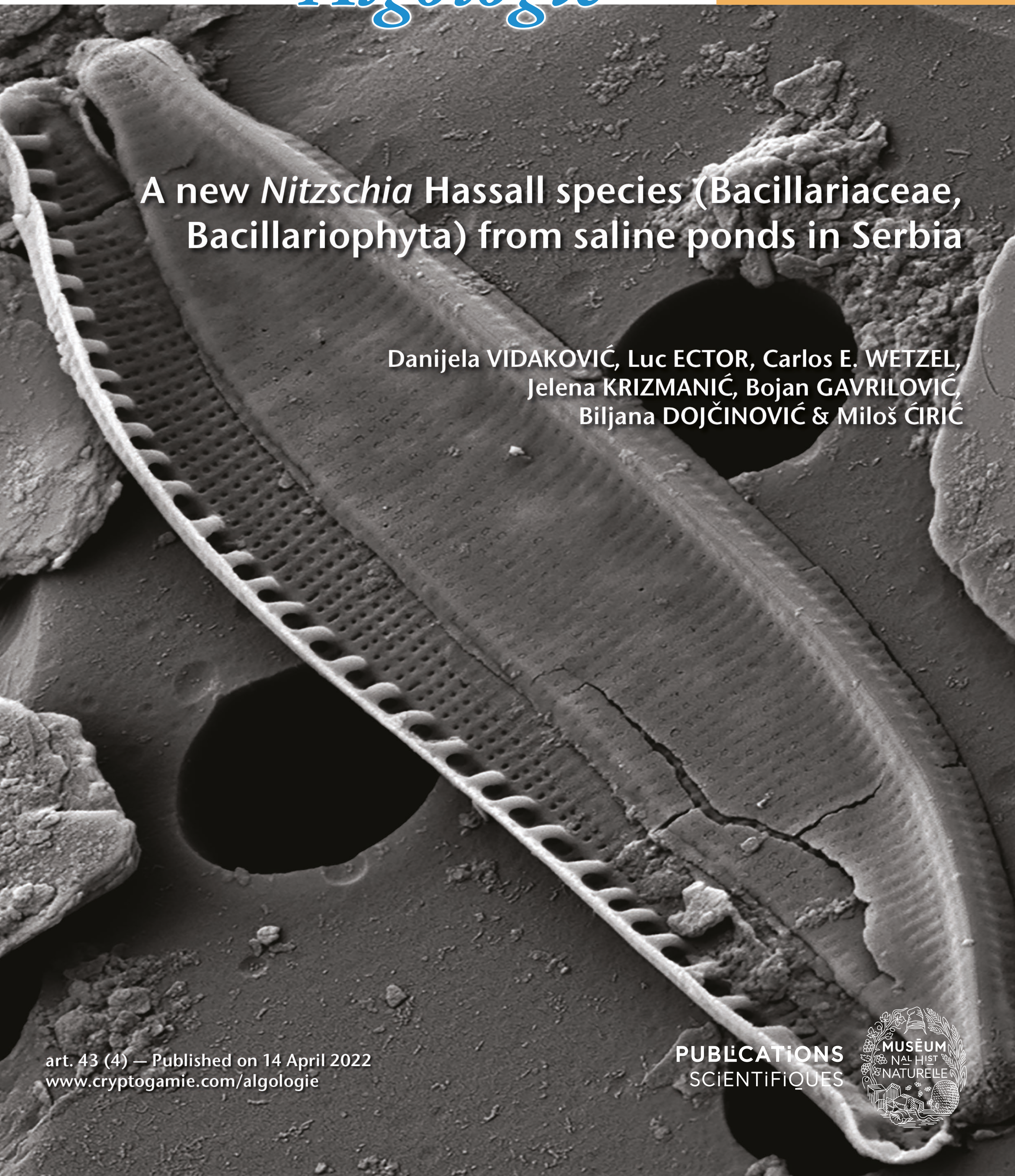

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A new *Nitzschia* Hassall species (Bacillariaceae, Bacillariophyta) from saline ponds in Serbia

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ABSTRACT
In the present study, a new species, *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov., is described from alkaline saline ponds and channels from Vojvodina Province (Serbia). In 2019 the new species was found in four saline ponds: Bela Bara, Čoka Kopovo, Velika Rusanda and Slatina. The same species was recorded four years ago in saline lakes in Hungary, but the authors listed it as *Nitzschia* sp. 2. The sigmoid valve outline of the new *Nitzschia* Hassall species is similar to *N. austriaca* Hust. but differs in striae density and fibulae distribution. In *N. haloserbica* sp. nov. the transapical striae are so dense that they are not visible in light microscopy. Both species have fibulae that are quite regularly distributed along the raphe canal, but in *N. austriaca* the central two fibulae are more widely spaced, reflecting the presence of central raphe endings, which are absent in *N. haloserbica* sp. nov. At the type locality (Bela Bara) both species were found with relative abundances of 94.93 % for *N. haloserbica* sp. nov. and 1.21 % for *N. austriaca* (of the 400 valves counting per slide). The valve morphology, ultrastructure and autecology of the new species are discussed and compared with morphologically similar taxa.

KEY WORDS
Bacillariales,
Bacillariaceae,
sigmoid outline,
saline habitats,
Bela Bara,
new species.

RÉSUMÉ
Une espèce nouvelle de Nitzschia *Hassall (Bacillariaceae, Bacillariophyta) provenant d'étangs salins en Serbie.* Dans la présente étude, une espèce nouvelle, *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov., est décrite provenant d'étangs et de canaux salins alcalins de la province de Vojvodina (Serbie). En 2019, l'espèce nouvelle a été trouvée dans quatre étangs salins : Bela Bara, Čoka Kopovo, Velika Rusanda et Slatina. La même espèce a été trouvée, il y a quatre ans, dans des lacs salins en Hongrie, mais les auteurs l'ont répertoriée comme *Nitzschia* sp. 2. Le contour de la valve sigmoïde de la nouvelle espèce de *Nitzschia* Hassall est similaire à celui de *N. austriaca* Hust. mais elle diffère par la densité des stries et la distribution des fibules. Chez *N. haloserbica* sp. nov., les stries transapicales sont si denses qu'elles ne sont pas visibles en microscopie optique. Les deux espèces ont des fibules assez régulièrement réparties le long du canal du raphé, mais chez *N. austriaca* les fibules centrales sont plus espacées, reflétant la présence de terminaisons centrales du raphé, qui sont absentes chez *N. haloserbica* sp. nov. Dans la localité type (Bela Bara), nous avons trouvé les deux espèces avec des abondances relatives de 94,93 % pour *N. haloserbica* sp. nov. et de 1,21 % pour *N. austriaca* (estimation à partir des 400 valves comptées par lame). La morphologie de la valve, l'ultrastructure et l'autécologie de la nouvelle espèce sont discutées et comparées à des taxons morphologiquement apparentés.

MOTS CLÉS
Bacillariales, Bacillariaceae, contour sigmoïde, habitats salins, Bela Bara, espèce nouvelle.

INTRODUCTION

Nitzschia is the second largest genus among diatoms. It was described by Hassall (1845: 435) and currently there are 1314 taxa names in AlgaeBase, although only 849 are taxonomically accepted (Guiry & Guiry 2021). It is one of the most widespread diatom genera, very common in fresh and brackish waters (Denys & Lange-Bertalot 1998; Trobajo *et al.* 2004), besides exhibiting high diversity in marine environments. Some of the main genus characters for diagnosis under the light microscope (LM) are finely structured valves with parallel striae, usually narrowly lanceolate valves with slightly protracted or subcapitate ends and almost invisible raphe (Trobajo *et al.* 2013). Specific morphometric features such as cell size, and stria and fibula density are also the principal diagnostic characters for delimitation of *Nitzschia* species. However, *Nitzschia* is a very heterogeneous genus in terms of symmetry, which includes one group of species with sigmoid valves, that may be common or even dominant in habitats with increased salinity (Ács *et al.* 2017, 2019).

The taxonomy of the genus *Nitzschia* is challenging due to the high species richness, the small number of features available for diagnosis in the light microscope, the morphological resemblance between some taxa, and the lack of ultrastructural knowledge (Lehmkuhl *et al.* 2019). In spite of these factors, the number of new species described has been increasing in recent years, as has the examination of type specimens, and documentation by electron microscopy (Tudesque *et al.* 2008; Trobajo *et al.* 2013; Hamsher *et al.* 2016; Ács *et al.* 2017; Carballeira *et al.* 2017; Lobo *et al.* 2017; Liu *et al.* 2017; Földi *et al.* 2018; Lehmkuhl *et al.* 2019; Lobban *et al.* 2019; Morales *et al.* 2020; Tan *et al.* 2020).

Usually, the genus *Nitzschia* is subdivided into several sections. In relation to valve shape, raphe position and fibula morphology Grunow distinguished 24 sections (Cleve & Grunow 1880). From that period, several simplifications of the Grunow's scheme have been proposed (Mann 1981; Mann *et al.* 2021). The biggest changes were made by Hustedt (1930) who simplified the classification of the sigmoid *Nitzschia* species. Sigmoid *Nitzschia* were classified by Grunow (Cleve & Grunow 1880) in four groups: *Sigmoideae* Grunow, *Sigmata* Grunow, *Obtusae* Grunow, and *Spectabiles* Grunow, although a few representatives of other groups (*Insignes*, *Nitzschiella*) are also sigmoid. Hustedt in 1930 merged the *Sigmoideae*, *Sigmata* and *Spectabiles* into the section *Sigmoideae* (Grunow) Hustedt. This section should be called section *Nitzschia* because it includes the *Nitzschia* generitype species, *N. sigmoidea* (Nitzsch) W.Sm. (Mann 1981, 1986). In Krammer & Lange-Bertalot (1988), Grunow's groupings were restored, giving three groups – *Sigmoideae*, *Sigmata* and *Obtusae* – the *Spectabiles* having meanwhile been transferred to *Hantzschia*. The latest research based on molecular data has shown that sigmoid *Nitzschia* species do not constitute a monophyletic group, and that they fall into three different clades which encompasses Grunow's Sigmoideae, Grunow's Sigmata, and Grunow's Obtusae species (Mann *et al.* 2021).

The purpose of this study is to illustrate one new sigmoid *Nitzschia* species from saline ponds in Serbia based on morphological observations with the light (LM) and scanning electron (SEM) microscope. The new species is compared with similar taxa and notes on its ecology and distribution are included.

MATERIAL AND METHODS

Study area and sample collection

This study was conducted in four saline ponds located in the southern part of the Carpathian Basin (the Vojvodina Region, Serbia) (Fig. 1). Diatom samples were collected between May and June 2019 from Bela Bara, Čoka Kopovo, Velika Rusanda, and Slatina ponds (Province of Vojvodina, Serbia), from submerged reed stems (*Phragmites australis* (Cav.) Trin. ex Steud.) and mud.

Preparation of slides and microscopy

Diatom samples were treated with hot concentrated HCl and supersaturated solution of $KMnO_4$ following the procedure described by Taylor *et al.* (2007). Permanent slides of the cleaned material were mounted with Naphrax (Brunel Microscopes Ltd). Light microscope observations and micrographs were made using a Zeiss AxioImagerM.1 microscope with differential interference contras (DIC) optics using a ×100 objective (Optovar 1.6× Magnification) and AxioVision 4.9 software. The abundance was estimated by counting 400 valves per slide. For SEM, parts of the oxidized suspensions were filtered with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum (30 nm) using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultra-high-resolution analytical field emission (FE) scanning electron microscope, Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan), operating at 5 kV and 10 mm distance, was used for the analysis. SEM images were taken using the lower (SE-L) detector signal and a tilting of up to 28°. Micrographs were digitally manipulated and plates containing light and scanning electron microscopy images were created using Adobe Photoshop CS6. The holotype slide is deposited in the Diatom Collection of Serbia (DCSR) at the University of Belgrade, Institute of Chemistry, Technology and Metallurgy. An isotype slide is deposited in the Meise Botanic Garden, Belgium.

Measurement of physicochemical parameters

In situ, water temperature (T), pH, and conductivity (COND) were measured using Water Multimeter 18.52.01 (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), while the dissolved oxygen (DO) was measured with a DO meter HI9147 (Hanna Instruments, Woonsocket, the USA). The determination of metal cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) was done by inductively coupled plasma optical emission spectrometry (ICP-OES) using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK). Ammonia concentration (NH_4^+, SRPS EN ISO 14911:2009), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) (U.S. EPA 1997), total phosphorus (TP, SRPS EN ISO 6878:2008), total nitrogen (TN, SRPS EN 12260:2008), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) (APHA, AWWA & WPCF, 1995a), chloride (Cl^-, APHA, AWWA and WPCF 1995b) and sulfate (SO_4^{2-}, APHA, AWWA, and WPCF 1995c) were measured in the laboratory. The Bela Bara and Slatina ponds were almost completely dry, thus water samples for chemical analyses of total cations and anions could not be taken.

RESULTS

Class BACILLARIOPHYCEAE Haeckel
Order BACILLARIALES Hendey
Family Bacillariaceae Ehrenb.
Genus *Nitzschia* Hassall

Nitzschia haloserbica
Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov.
(Figs 2; 3)

Nitzschia sp. 2 – Stenger-Kovács & Lengyel 2015: 144-145, pl. 68, figs 1-24.

Nitzschia haloserbica Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov. can be distinguished from other sigmoid *Nitzschia* species, in particular *N. austriaca* by stria density, and central fibulae. In *N. austriaca* the transapical striae are visible in LM, and central fibulae are more widely spaced reflecting the presence of central raphe endings, which are absent in *N. haloserbica* sp. nov.

Holotype. — **Serbia**. Bela Bara saline pond, 45°56'50.6796"N, 19°5'25.7676"E, 08.V.2019, Leg. D. Vidaković, (accession no. DCSR 000062) (holo-, ICTM[slide DCSR 000062/A]; iso-, BR[slide BR 4599 Meise Botanic Garden, Meise, Belgium]). The holotype is marked drawing an ink ring with a fine waterproof felt pen on the cover slip and is illustrated in Fig. 2BI.

Etymology. — The specific epithet "halo" indicates that the species is characteristic for saline habitat and "serbica" that is found in Serbia.

Distribution. — *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov. was recorded in four saline ponds in Serbia (Fig. 1). The species was found in epipelic (Bela Bara, Čoka Kopovo, and Slatina ponds) and epiphytic communities (Čoka Kopovo, Velika Rusanda, and Slatina ponds). The higher relative abundance of this species was on mud (epipelic material), at the type locality of Bela Bara pond (94.93 %). In Čoka Kopovo, Velika Rusanda, and Slatina ponds the relative abundance was lower (8.98 %, 0.99 %, 0.65 %, respectively). In Hungary, this species was named as *Nitzschia* sp. 2 and was recored in 7 saline lakes (Böddi-szék, Büdös-szék, Fehér-szék, Kardoskúti Fehér-tó, Kele- men-szék, Kisréti-tó, Zab-szék) with mean/max. abundance 22.7 %/72.2 % (Stenger-Kovács & Lengyel 2015).

Ecology. — The investigated saline ponds are shallow water bodies with high pH, except for the Slatina which is a degraded saline pond (Table 1). According to Hammer's (1986) salinity classification, Velika Rusanda pond can be characterized as hyposaline, while Čoka Kopovo pond fitted into the subsaline category. Depending on the different combinations and order of dominant anions existed in water Velika Rusanda pond can be classified as Na-HCO_3-SO_4-Cl and Čoka Kopovo pond as sodic base type (Na-HCO_3). Data found in previous papers as well as determined by subsequent surveys of Bela Bara and Slatina ponds have been shown that Slatina pond fitted into the subsaline category white dominance of sodium and magnesium (Na-Mg-HCO_3) (Ćirić *et al.* 2021), while Bela Bara can be classified as sodic base type (Na-HCO_3) (Boros *et al.* 2014).

Description

Light microscope (Fig. 2)

Valve linear, strongly curved towards opposite sides (sigmoid), progressively tapering at some distance from the slightly protracted and sometimes subcapitate ends. Frustules are sigmoid in valve and girdle view. Valve length 14.3-46.4 µm, width 3.1-3.6 µm (dimensions of population in Bela Bara: length

FIG. 1. — Location of the four study saline ponds in the Vojvodina Region, Serbia. **A**, Bela Bara; **B**, Čoka Kopovo; **C**, Velika Rusanda; **D**, Slatina.

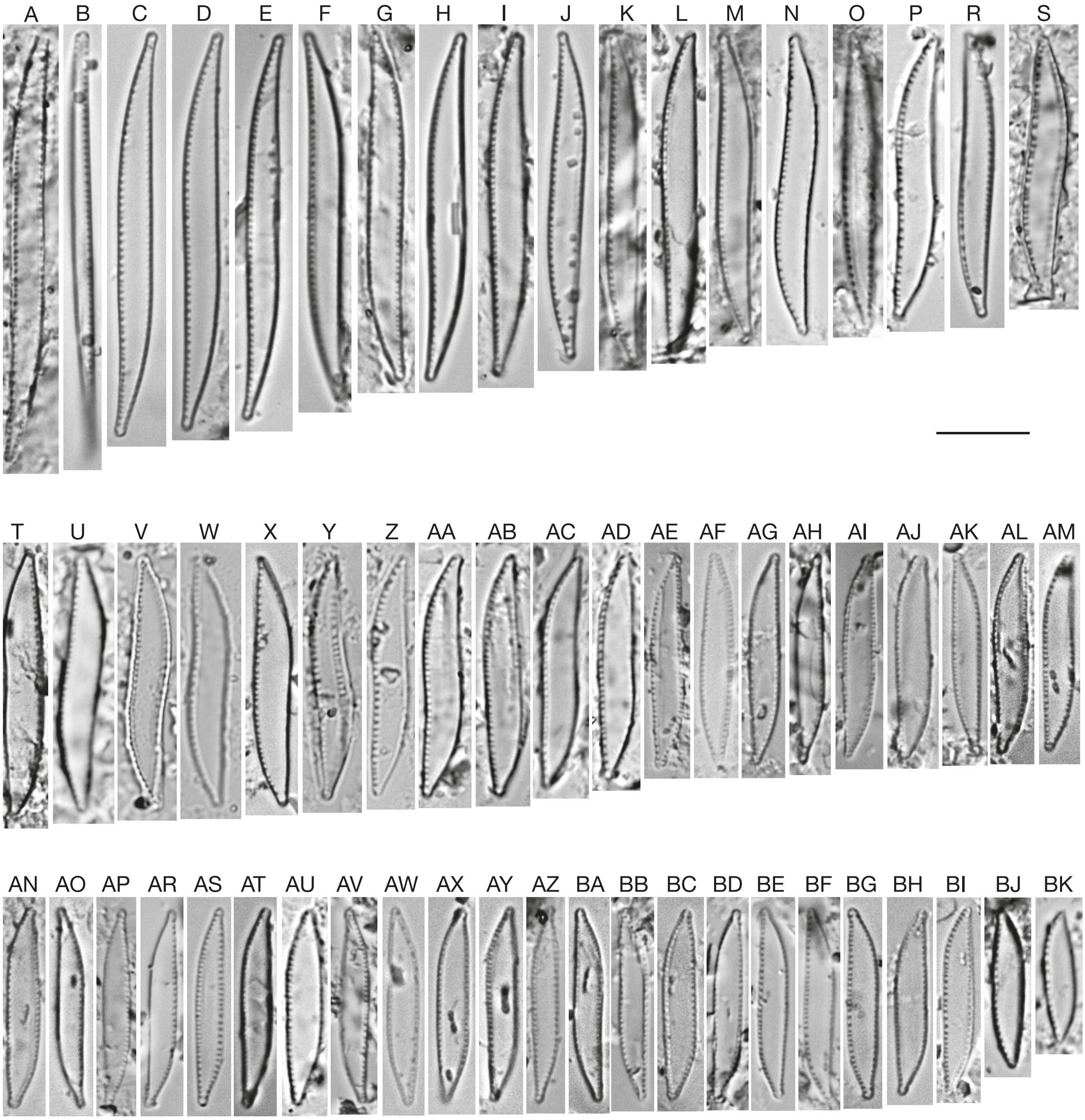

FIG. 2. — *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov.: **A**-**BK**, LM valve views of the type population (Bela Bara saline pond) arranged in decreasing length. Scale bar: 10 µm.

16.8-34 µm, width 3.1-3.5). Fibulae quite regularly distributed along the raphe canal, the central pair not more widely separated, 12-18 in 10 µm. Transapical striae not visible in LM.

Scanning electron microscope (Fig. 3)
The striae are uniseriate, 54-75 in 10 µm. Areole circular, slightly domed hymenes close to the external apertures of the areolae (Fig. 3E). Two single rows of areolae surround the keel, and never doubled on the raphe keel. An inner valve view details tubular to rectangular fibulae quite regularly distributed along the raphe canal. The raphe is eccentric. The distal raphe endings straight. No central nodule. A small helictoglossa is present internally at distal raphe endings. On the mantle, the striae are very short, comprising one areola (Fig. 3B).

DISCUSSION

Nitzschia haloserbica Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov. is common, sometimes dominant sigmoid *Nitzschia*

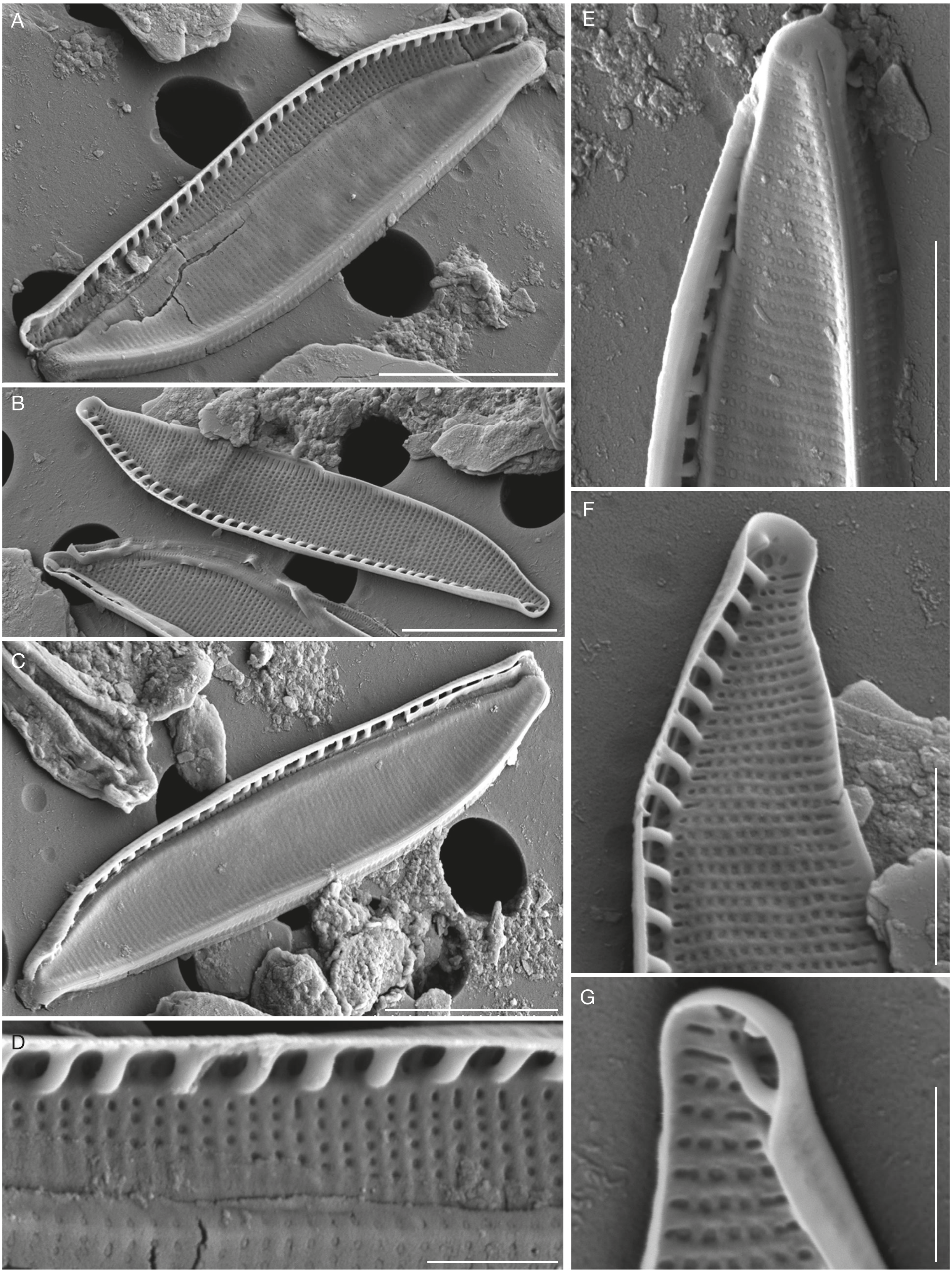

Fig. 3. — *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov., SEM images taken from the type material (Bela Bara saline pond): **A**, **C**, **E**, SEM views of a frustule in valve view; **B**, SEM internal view of two valves from the same frustule; **D**, SEM internal view of the center of the valve with fibulae regularly distributed; **F**, SEM internal view of valve apex with helictoglossa and raphe; **G**, SEM internal detail view of valve apex with helictoglossa. Scale bars: A-C, 5 µm; D, G, 1 µm; E, 3 µm; F, 2 µm.

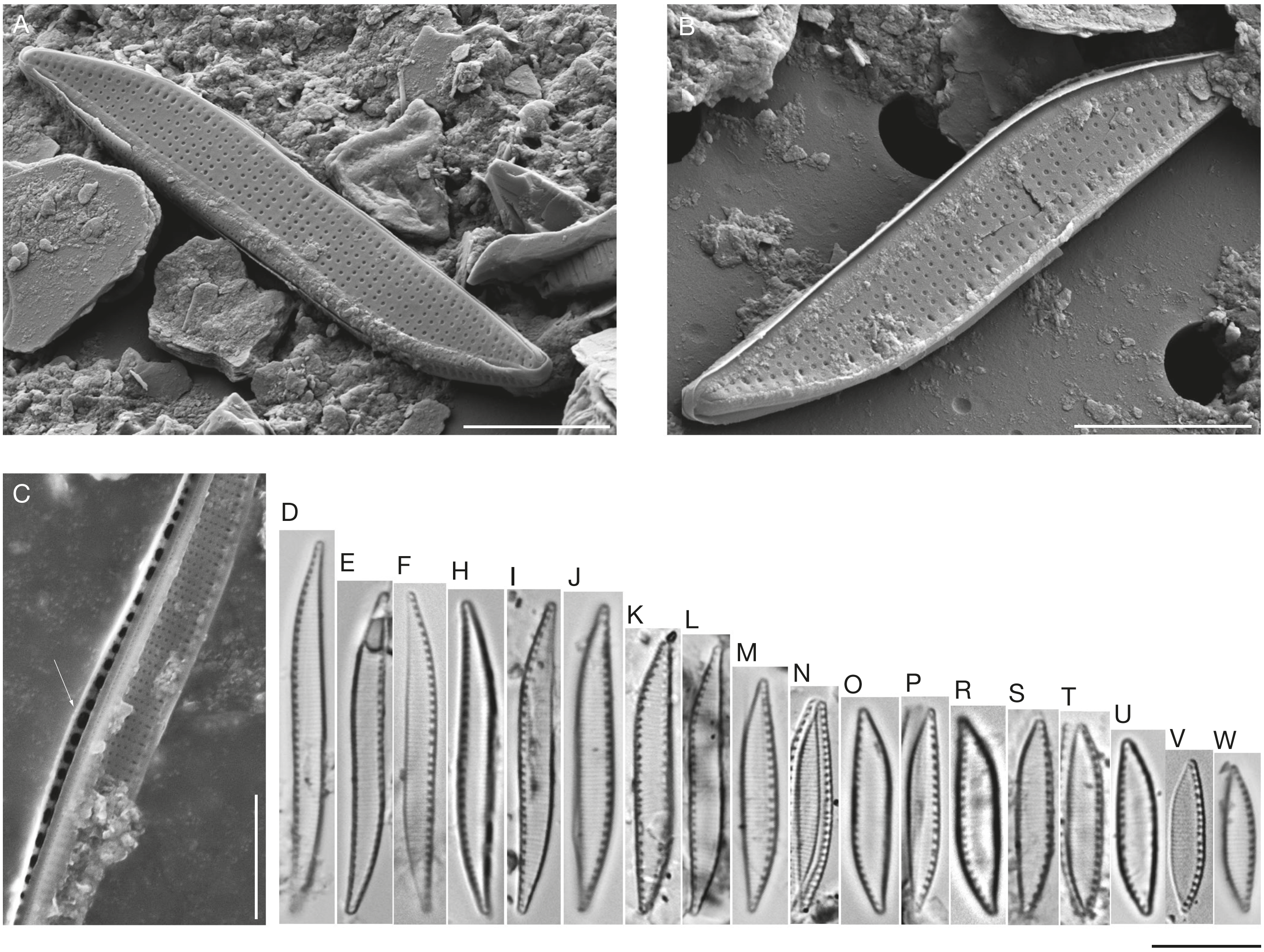

FIG. 4. — *Nitzschia austriaca* Hust. SEM and LM images taken from Bela Bara saline pond. **A**-**C**, SEM external valve views of a frustule; **C**, arrow indicates more widely spaced center fibulae; **D**-**W**, LM valve views of a population arranged in decreasing length. Scale bars: A-C, 5 µm; D-W, 10 µm.

species in Serbian saline ponds. Stenger-Kovács & Lengyel (2015) introduced this species as a possibly new taxon requiring further formal identification in the *Taxonomic and distribution guide of diatoms in soda pans of Central Europe*, identified as *Nitzschia* sp. 2 (Stenger-Kovács & Lengyel 2015: pl. 68, figs 1-24). They noted that the species is similar to *Nitzschia austriaca* Hust. (Hustedt 1959: 439, figs 28-31), but it is wider and differs in fibula and stria density (Stenger-Kovács & Lengyel 2015). Populations recorded in Serbian saline ponds has a larger range in valve length (14.3-46.4 µm toward 19.6-22.2 µm), but a smaller range in the fibulae densities (12-18 per 10 µm toward 6-19 per 10 µm). However, width varying little within a population (3.1-3.6 µm toward 2.8/3.5 µm).

According to Krammer & Lange-Bertalot (1988), the *Sigmoideae* group includes the large-sized species, sigmoid only in girdle view, and *Nitzschia haloserbica* sp. nov., sigmoid in valve and girdle view, does not belong to this group. The species belonging to the group *Sigmata* are sigmoid in both valve and girdle views, with the strongly eccentric raphe system (keel). On the other hand, the *Obtusae* section may be sigmoid in girdle view only, in valve view only, or in both. Furthermore, in *Obtusae* the raphe system may be anything from nearly central to submarginal and it does not appear keel-like. Finally, this group is set apart from the other groups by lack of the conopeum characteristic of *Sigmoideae* and the presence of deflected central raphe endings (Mann 1981; Krammer & Lange-Bertalot 1988).

Nitzschia austriaca, which belongs to the *Sigmata* group, is similar to the new species, but clearly differs in relation to valve ends (more capitate ends than in *N. austriaca*), valve width, stria density and central fibulae (central nodule present). In our samples *Nitzschia haloserbica* sp. nov. is recorded together with *N. austriaca* (Fig. 4), except in the Slatina saline pond. In the type locality (Bela Bara pond) the new species was very abundant with 94.93 % (of the 400 valves counting per slide), while *N. austriaca* had only an abundance of 1.21 %. A higher dominance of *N. austriaca* was noted in Čoka Kopovo pond, in the epiphytic community (30.42 % against 8.98 %), while the relative abundance of both species was less than 1 % in Velika Rusanda pond. In *N. haloserbica* sp. nov. transapical striae are not visible in LM. In addition, in both species the fibulae are quite regularly distributed along the raphe canal, but in *N. austriaca* central fibulae are more widely spaced than the others.

Table 1. — Physical and chemical characteristics of water in four Serbian saline ponds sampled in 2019. **ND**, no data available.

Parameters/Ponds	Bela Bara	Čoka Kopovo	Velika Rusanda	Slatina
Temperature (°C)	21.1	25.7	32.7	30.4
pH	9.63	8.21	9.60	7.67
Conductivity (µS/cm)	7565	813	14300	828
Dissolved oxygen (mg/L)	ND	7.20	7.20	ND
Potassium (mg/L)	ND	7.12	140.80	ND
Sodium (mg/L)	ND	163.5	2908.0	ND
Calcium (mg/L)	ND	34.28	6.20	ND
Magnesium (mg/L)	ND	4.36	8.22	ND
p-alkalinity (ml 0.1N HCl)	ND	21.2	219.2	ND
m-alkalinity (ml 0.1N HCl)	ND	92.7	446.2	ND
Carbonate (mg/L)	ND	127.2	1315.2	ND
Bicarbonate (mg/L)	ND	306.8	47.6	ND
Ammonia (mg/L)	ND	1.35	1.71	ND
Nitrite (mg/L)	ND	< 0.020	0.390	ND
Nitrate (mg/L)	ND	0.80	< 0.5	ND
Total nitrogen (N mg/L)	ND	1.4	1.5	ND
Phosphate (mg/L)	ND	0.487	21.00	ND
Total phosphorus (P mg/L)	ND	0.560	23.00	ND
Chloride (mg/L)	ND	63.7	2161.0	ND
Sulphate (mg/L)	ND	8.1	2401.0	ND

Table 2. — Comparison of morphological features of *Nitzschia haloserbica* Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov. and related sigmoid *Nitzschia* species. *, measured in populations from Bela Bara, Čoka Kopovo and Velika Rusanda saline ponds.

Taxa	Length (µm)	Width (µm)	Striae in 10 µm	Fibulae in 10 µm	Reference
Nitzschia haloserbica Vidaković, Ector, C.E.Wetzel & Krizmanić, sp. nov.	14.3-46.4	3.1-3.6	not visible	12-18	this study
Nitzschia adiembraensis Foged	35-40	4	not visible	11	Foged 1966
Nitzschia aremonica R.E.M.Archibald	(31) 38-75	4-5	24-28	7-10	Archibald 1983
Nitzschia austriaca Hustedt	*14.5-34.5	2.7-3.4	26-30	11-15	*
	12-22	1.8-2.4	*c.* 26	*c.* 12	Hustedt 1959
	13.6-34	2.4-3.3	26-30	12-16	Ács *et al*. 2017
Nitzschia brevissima Grunow	18-54	3.5-6.5	30-38	5-10	Lange-Bertalot *et al*. 2017
Nitzschia clausii Hantzsch	20-55	3-5	38-42	10-13	Lange-Bertalot *et al*. 2017
Nitzschia filiformis var. *conferta* (Richt) Lange-Bertalot	20-45	3-5	27-32	7-11	Krammer & Lange-Bertalot 1988
Nitzschia maillardii M.Coste & Ricard	35-40	4	not visible	10-11	Coste & Ricard 1982
Nitzschia nana Grunow	35-120	3-4.5	30-36	7-11	Lange-Bertalot *et al*. 2017
Nitzschia obtusa var. *scalpelliformis* f. *lata* (Grunow) Grunow	23.5	5	24	14	Guermeur 1954
Nitzschia obtusa var. *scalpelliformis* f. *nipponica* (Grunow) Grunow	17-40	3-4	not visible	9-11	Negoro 1944
Nitzschia parvula var. *terricola* J.W.G.Lund	25-70	4-5	31-37	6-9	Lund 1946
Nitzschia parvuloides f. *curta* Cholnoky	20	4	34	7-8	Cholnoky 1954
Nitzschia rotula J.R.Carter	25-56	4	38-42	7-10	Carter 1966
Nitzschia terrestris (J.B.Petersen) Hustedt	24-115	3-5	32-35	5-8	Krammer & Lange-Bertalot 1988
Nitzschia torta H.P.Gandhi	36-40	3.3-3.8	18-20	7-8	Gandhi 1961

Nitzschia haloserbica sp. nov. can be clearly distinguished from other similar sigmoid *Nitzschia* Hassall. *Nitzschia brevissima*, *N. clausii*, *N. filiformis* var. *conferta*, *N. nana*, *N. terrestris*, and *N. torta* have significantly less fibulae and less striae density unlike *N. haloserbica* sp. nov. (54-74 in 10 µm) (Table 2). Also, *N. clausii* has central nodule, while central nodule has not observed in *N. haloserbica* sp. nov. and proximal raphe ends of *N. clausii* curve toward the valve centre. Compared to *N. haloserbica* sp. nov., whose valve width is in the range 3.1-3.6 µm and number of fibulae 12-18 in 10 µm, *N. adiembraensis*, *N. aremonica*, *N. maillardii*, *N. obtusa* var. *scalpelliformis* f. *lata*, *N. parvula* var. *terricola*, *N. parvuloides* f. *curta*, and *N. rotula* have wider valves and less fibulae (Table 2). *Nitzschia obtusa* var. *scalpelliformis* f. *nipponica* has the same high striae density (not resolvable with LM) as *N. haloserbica* sp. nov., but less fibulae (9-11 in 10 µm) than the new species (12-18 in 10 µm).

Accurate identification is crucial, since many species of the genus *Nitzschia* have been shown to be important indicators of environmental conditions (Lengyel *et al.* 2016). Stable morphological characters of the new species and the fact that it can be easily distinguished from the others sigmoid *Nitzschia* can have significance in the further ecological studies and biomonitoring in Central European saline lakes.

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