

Nitzschia taikiensis sp. nov. (Bacillariophyta) – a new brackish diatom from the Toberi River marsh in the eastern Hokkaido, Japan

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Abstract. *Nitzschia taikiensis* sp. nov. is a brackish diatom species found in the Toberi River marsh, eastern Hokkaido, Japan. This species has characteristics similar to *Nitzschia subamphioxoides* Hustedt, which was originally described by Hustedt in 1959. In the present study, we conducted a comparative morphological analysis of *N. taikiensis* and *N. subamphioxoides*. The obtained results showed that they are different species. Sampling sites of *N. taikiensis* were located in salt marsh environments with very low salinity (1-5‰), acidic pH (5.2-5.9) and high mud content (95.0-97.5%). Identification of this species was relatively easy based on LM and SEM image analysis of its frustule features, such as the external form and stria density. This species has not been previously reported in Japan, which could be attributed to the reduction or loss of Japanese freshwater and salt marsh environments in the coastal areas, resulting from urban and industrial development.

Key words: diatom, new species, *Nitzschia subamphioxoides*, ecology, Hokkaido, very low salinity environment

1. Introduction

Many diatom species inhabit brackish water environments and comprise an important part of the ecosystem. Species of the genus *Nitzschia* Hassall are some of the diatom species that are typically found in brackish water environments (Denys & Lange-Bertalot 1998). Many species of this genus are important environmental or paleo-environmental indicators (Vos & de Wolf 1993; Van dam *et al.* 1994; Chiba & Sawai 2014). In addition, the genus *Nitzschia* comprises a large number of species. Since its first description by Hassall (1845), new species of *Nitzschia* continue to be discovered; moreover, reclassifications of the members of this genus have been frequently conducted in recent years (e.g., Liu *et al.* 2017). The microstructure of frustules is in many cases important for the identification of members of this genus and detailed observations are often required (Round *et al.* 1990).

An investigation conducted on 25th April 2017 revealed a relatively high abundance of *Nitzschia* sp. in the low salinity, brackish water environments of the Toberi River marsh, Hokkaido, northern Japan (Fig. 1). An initial light microscope (LM) image analysis of this *Nitzschia* sp. showed that it is similar to *Nitzschia subamphioxoides* Hustedt, one of *Nitzschia* species originally described and sketched by Hustedt (1959a) as *Nitzschia amphioxoides*. This species was subsequently renamed *N. subamphioxoides* by Hustedt (1959b). Its photographs are included in Simonsen (1987). *Nitzschia subamphioxoides* was first discovered in a very low salinity environment in Salzlackengebietet, Burgenland, Austria. Since then, this species has been reported in very few locations around the world, e.g., Lake Okoboji and Lazy Lagoon in Iowa (freshwater lakes in America) by Stoermer (1963), Great Lakes (freshwater lakes in America) by Stoermer *et al.* (1999), and Petuniabukta (Spitsbergen) by Pinseel *et al.* (2017).

Moreover, although *N. subamphioxoides* was described using LM image analysis by Simonsen (1987), there is currently no description of this species based on electron microscope (SEM) image analysis. When preparing the description of *Nitzschia* sp. nov. we studied the typical material of *N. subamphioxoides* and took both LM and SEM photographs for comparative purposes, which are included in this paper.

Nitzschia sp. probably prefers brackish water marsh environments and low salinity because this species has not been found in any other environment of the Toberi River area (Chiba & Nishimura 2018). In this study, we described *Nitzschia* sp. as a new species for science – *Nitzschia taikiensis* sp. nov., and compared *N. taikiensis* and *N. subamphioxoides* and other *Nitzschia* spp. using LM and SEM image analysis. Some studies recognized *N. subamphioxoides* as a synonym of *Nitzschia commutata* Grunow (Krammer & Lange-Bertalot 1988; Witkowski *et al.* 2000; Hamsher *et al.* 2016). We also examined the possibility that the studied *Nitzschia* sp. can be either a previously described or novel species.

2. Study area

Many coastal areas in Japan are protected by revetments and the coastal area of Hokkaido is one of a

few natural wetlands remaining. A vast marsh occupies the mouth of the Toberi River in Taiki, Hokkaido, Japan (Fig. 1). Taiki is one of the cities located in the eastern part of Hokkaido. This marsh is at a straight-line distance of 3 km inland from the coast. The sea side of this marsh is separated by sandbars from the Pacific Ocean. A previous study reported changes in fossil diatom assemblages near this area during the last millennium (Chiba *et al.* 2018).

3. Material and methods

The abundance of living and dead diatoms was surveyed in the field on 25 April 2017. Five samples were obtained (MS1-MS5) using 10-cc syringes and fixed in 5% formalin (Fig. 1). The latitude/longitude and elevation of sampling sites were measured using a GPS device (ProMark 3; Magellan America, Potpourri Group, North Billerica, Massachusetts, U.S.A.; and GEM-1, GNSS Technologies, Inc., Tokyo, Japan) and kinematic survey methods. At each site, the salinity was measured with a Salinity Refractometer (ATC-S/Mill-E; ATAGO, Tokyo, Japan) and the pH, electrical conductivity (EC), and oxidation-reduction potential (Eh) were measured with a portable pH/EC meter (D-54; Horiba, Kyoto, Japan). Each sample was dried

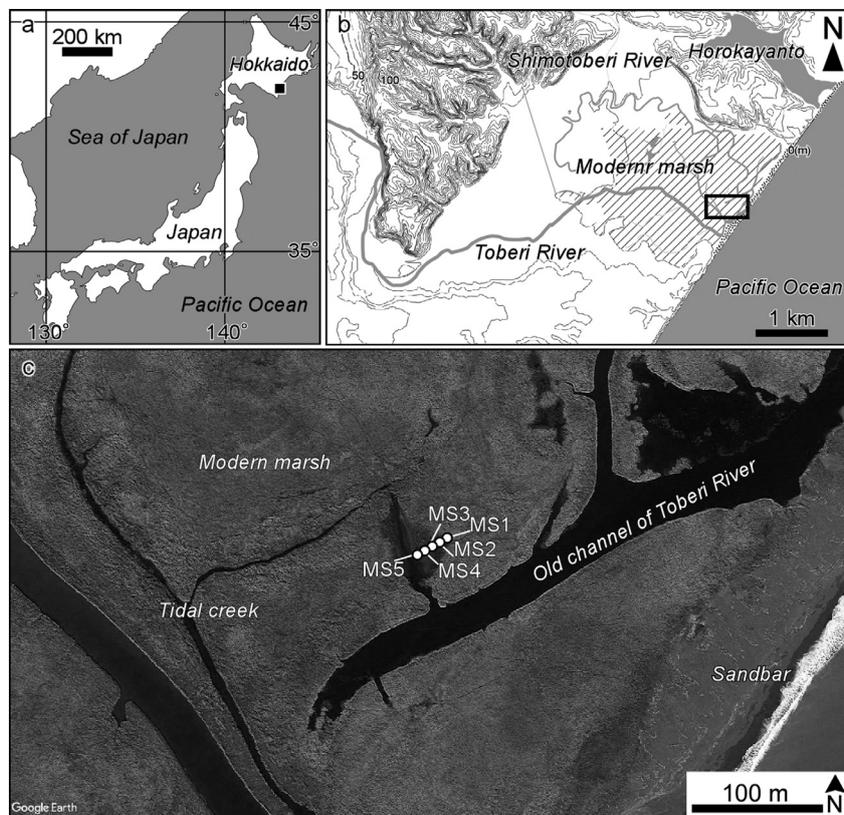


Fig. 1. Study area and sampling sites

Explanations: a – location of the study area in Hokkaido, Japan (black square); b – location of the Toberi River marsh (rectangle) – a contour map based on Tani (2015) and GSI Tiles (elevation tiles: <http://maps.gsi.go.jp/development/ichiran.html>); c – Google Earth aerial photograph (2016) showing the location of sampling sites (MS1-MS5)

at 110°C for 24 h and analysed to estimate organic matter contents in the bottom sediments using the loss on ignition (LOI) method at 750°C for 1 h (Japanese Standards Association 2000). Plant materials were removed from the bottom sediments of each sample and after H₂O₂ treatment, the sand and mud contents were measured with a laser diffraction particle size analyser (SALD-3000S; Shimadzu, Kyoto, Japan).

To assess the diatoms in the samples (MS1-MS5), the formalin fixed samples were rinsed in pure water in the laboratory, pre-treated with haematoxylin-eosin stain (Kosugi 1985; Sawai 2001) to distinguish between living and dead cells, and mounted on slides with Pleurax medium (Mount Media; Wako, Osaka, Japan). The sample of the *N. subamphioxoides* type material was cleaned by H₂O₂ treatment. The prepared slides were examined under a LM at 600× and 1000× magnification with an oil immersion objective. At least 700 valves of diatoms were counted on each LM slide (MS1-MS5)

and the relative abundance was calculated. For SEM analysis, frustules of the diatoms were cleaned by H₂O₂ treatment and coated with gold.

4. Results

Nitzschia taikiensis Chiba, Nishimura, Horie et Tuji sp. nov.

Holotype: marked specimen in slide TNS-AL-58962sa in the Collection of TNS (Department of Botany, National Museum of Nature and Science) represented by Fig. 2e-f.

Isotype (raw material): TNS-AL-58962m (Fig. 2).

Type locality: Toberi River marsh, Taiki, Hiroo, Hokkaido, Japan (Fig. 1).

Description: Frustules in girdle view slightly rectangular with rounded corners, slightly constricted in the middle, with girdle composed of numerous bands,

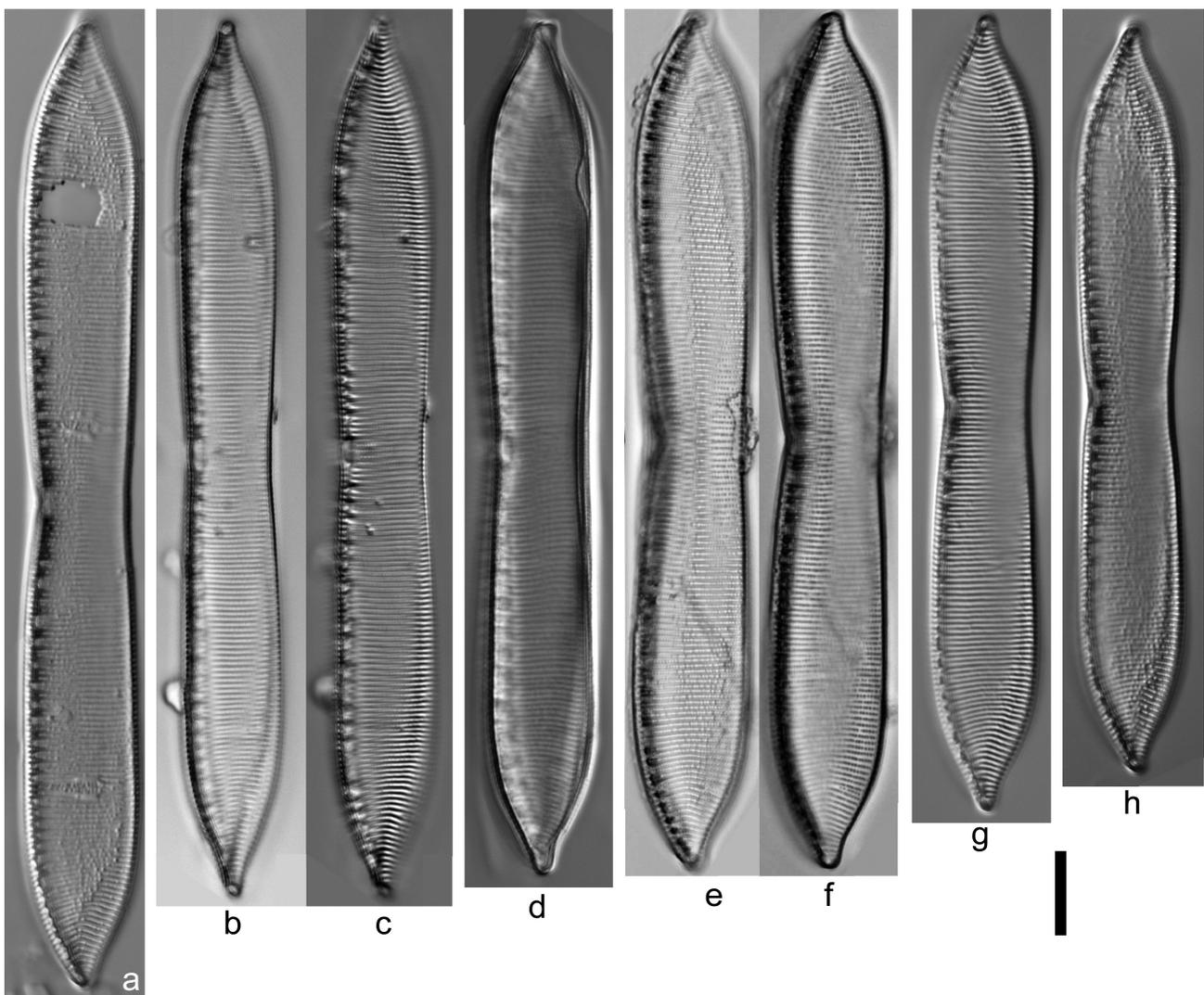


Fig. 2. LM images of the isotype and holotype specimens of *Nitzschia taikiensis*

Explanations: a-h – valve views showing size range, b-c – images of the same individual, e-f – represent the holotype (images of the same individual), scale bar = 10 μm

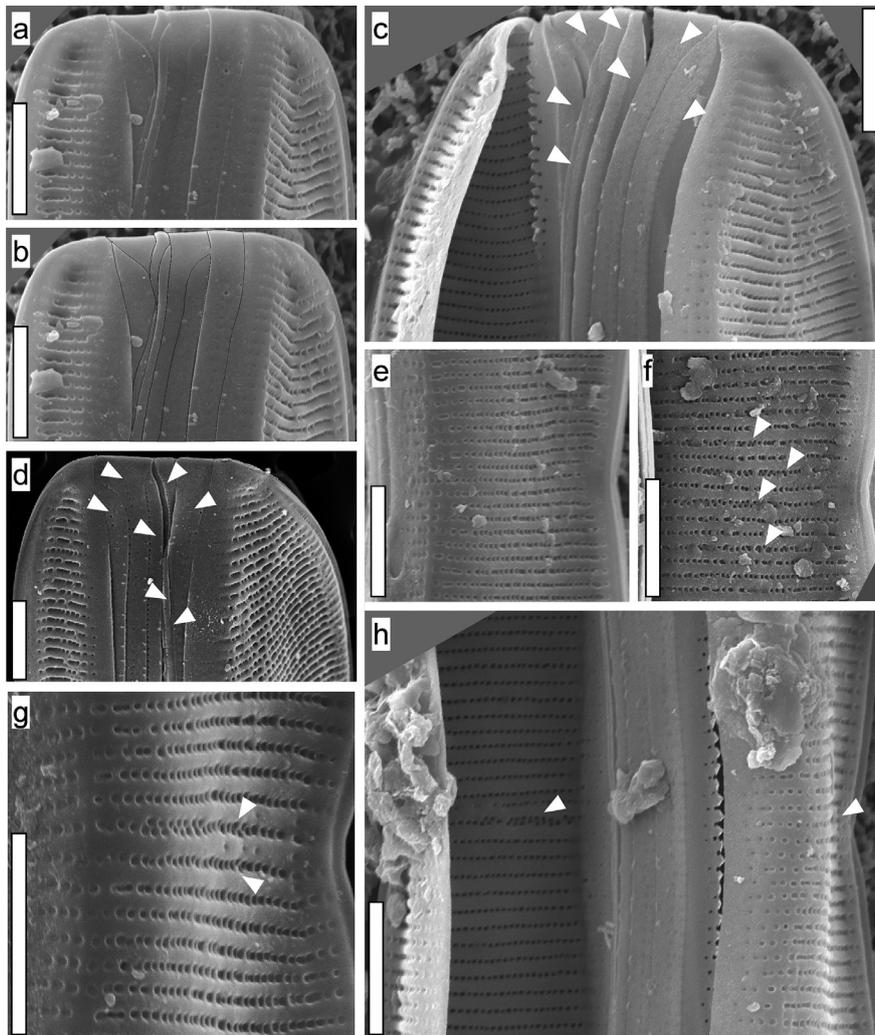


Fig. 3. SEM images of microstructures in *Nitzschia taikiensis* – bands and areolae

Explanations: a – girdle polar view showing 7 bands, b – outlines of bands were traced with black lines, c – oblique polar view showing 7 bands (arrow heads), d – girdle polar view showing 7 bands (arrow heads), e – external view of the central area showing areolae, f–g – internal views of the central area showing areolae and double areolae, h – internal, oblique view showing areolae and double or tripled areolae (arrow heads), scale bar = 5 μm

usually 7 (5–7 bands) with small pores on the advalvar side were observed in SEM (Fig. 3h), the open ends of the bands occurred at alternate ends of the frustule (Fig. 3c). Valves linear, slightly panduriform, with somewhat produced cuneately narrowed rostrate to capitate ends, 70–160 μm long, 7–13 μm wide. Canal raphe strongly eccentric, in the middle constricted. Transapical striae easily resolvable under LM, parallel throughout the whole valve, becoming radiate only at the apices, 15–20 in 10 μm . Fibulae 5–9 in 10 μm , the two middle a little further apart ($n=50$). LM images of the cytoplasm and nuclei, dyed with haematoxylin-eosin stain, of the new species are illustrated in Fig. 4.

The diatoms were, on average 95 μm in length, 9 μm in width, with an average of 16/10 μm striae, 21/10 μm areola and 7/10 μm fibulae ($n=50$). The valve face was arch shaped with a smaller curvature and a higher central area in the keel sides, and had a wave shape (this feature was clearer in the SEM images). The striae were

arranged in parallel and their density did not change from the valve center to the valve edge, however, they were slightly curved near the edge. One or two striae were inserted between two fibulae and the spacing was heterogeneous. The central nodule was clearly recognized at one side of the valve edge.

SEM analysis: External valve surface convex, with curvature decreasing towards the valve margins (Fig. 5). Canal raphe surface decorated with a single row of areolae conforming to those of the striae forming (Fig. 6g). Only in the valve middle, there were observed two areolae on the canal raphe (Fig. 6g). Externally raphe filiform, on a somewhat elevated raphe sternum, raphe branches straight with distinct external and internal proximal ends (central nodule). External proximal raphe ends simple and somewhat distant from each other, internal proximal raphe ends simple, close to each other and separated by a small double helictoglossa. External apical raphe ends strongly hooked to one side

and internally terminate in a distinct helictoglossae. Transapical striae uniseriate, composed of transapically elongated irregularly spaced areolae. Internally the areolae are regularly spaced and circular, 29-48/10 μm (mean: 40/10 μm). In the valve middle two- to tri-seriate rows of areolae were observed (Figs. 3, 6). Fibulae plain, irregular in shape, appearing fairly massive, 5-9 in 10 μm with the two middle ones somewhat distant from each other.

Ecology: The environmental data measured at sampling sites are shown in Table 1. Elevations of sampling sites were 0.6-1.5 m above sea level (asl), and the bottom sediment was peat (Table 1). The brackish waters of river estuary, with moderately high (or medium) electrolyte content, weakly acidic and eutrophic (Table 1). *Nitzschia taikiensis* was recognized in all sampling sites (MS1-MS5). Furthermore, many *Chamaepinnularia* sp., *Melosira* sp., *Navicula*

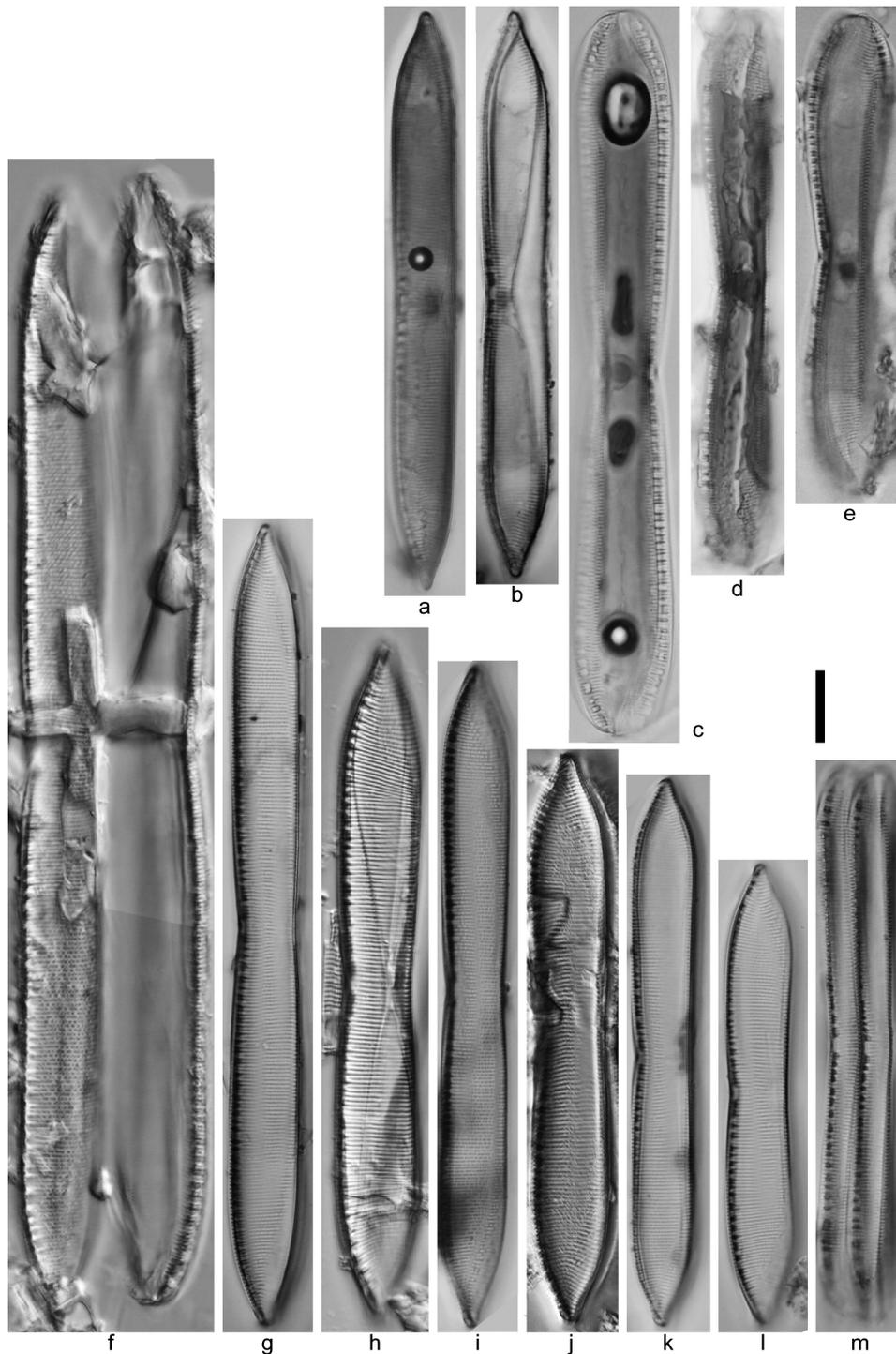


Fig. 4. LM images of the living and dead cells of *Nitzschia taikiensis*

Explanations: a-e – living cells (dyed), f-m – dead cells, a-b and f-l – valve views, c-e and m – girdle views, scale bar = 10 μm

Table 1. Data on the studied locations and water and bottom sediment parameters in the sampling sites of *Nitzschia taikiensis*

	MS1	MS2	MS3	MS4	MS5
Sampling date	April 25th, 2017				
Environment	Salt marsh				
Bottom sediment	Peat	Peat	Peat	Peat	Peat
Longitude	143.460965	143.460896	143.460819	143.460735	143.458897
Latitude	42.511987	42.511956	42.511921	42.511883	42.507469
Elevation (m asl)	1.5	1.4	1.4	1.2	0.6
Sand content (%)	2.5	2.4	3.6	5.0	5.0
Mud content (%)	97.5	97.6	96.4	95.0	95.0
LOI (%)	70.2	62.1	57.0	52.3	53.4
EC (mS/m)	18.8	19.1	23.8	24.3	24.0
Salinity (‰)	1	2	3	5	5
pH	5.2	5.2	5.9	5.8	5.9
Eh (mV)	321	319	312	300	289
Relative abundance of living cells (%)	4.6	5.0	0.9	0.7	0.5
Relative abundance of dead cells (%)	1.9	5.5	0.5	0.5	0.7

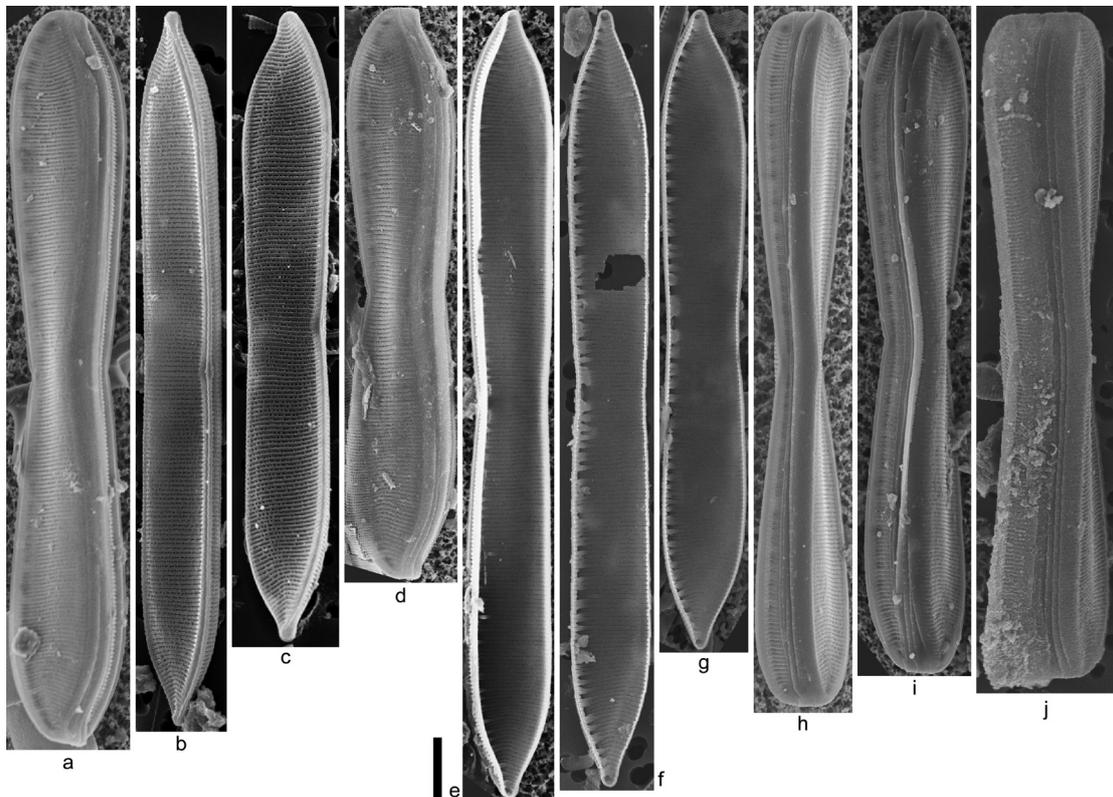
Explanations: LOI – loss on ignition, EC – electrical conductivity, Eh – redox potential

peregrine (Ehrenberg) Kützing, *Pinnunavis elegans* (W. Smith) Okuno, *P. elegantoides* (Hustedt) Cocquyt et Olodo and *Pseudostaurosira brevistriata* (Grunow) D.M. Williams were also identified in each sample.

E t y m o l o g y : The species name refers to the city

of Taiki, where the samples with a new species were collected.

***Nitzschia subamphioxoides* Hustedt, Österr. Bot. Z. 106: 415. 1959.**

**Fig. 5.** SEM images of *Nitzschia taikiensis*

Explanations: a-d – external views, e-g – internal views, h-j – girdle views, scale bar = 10 μ m

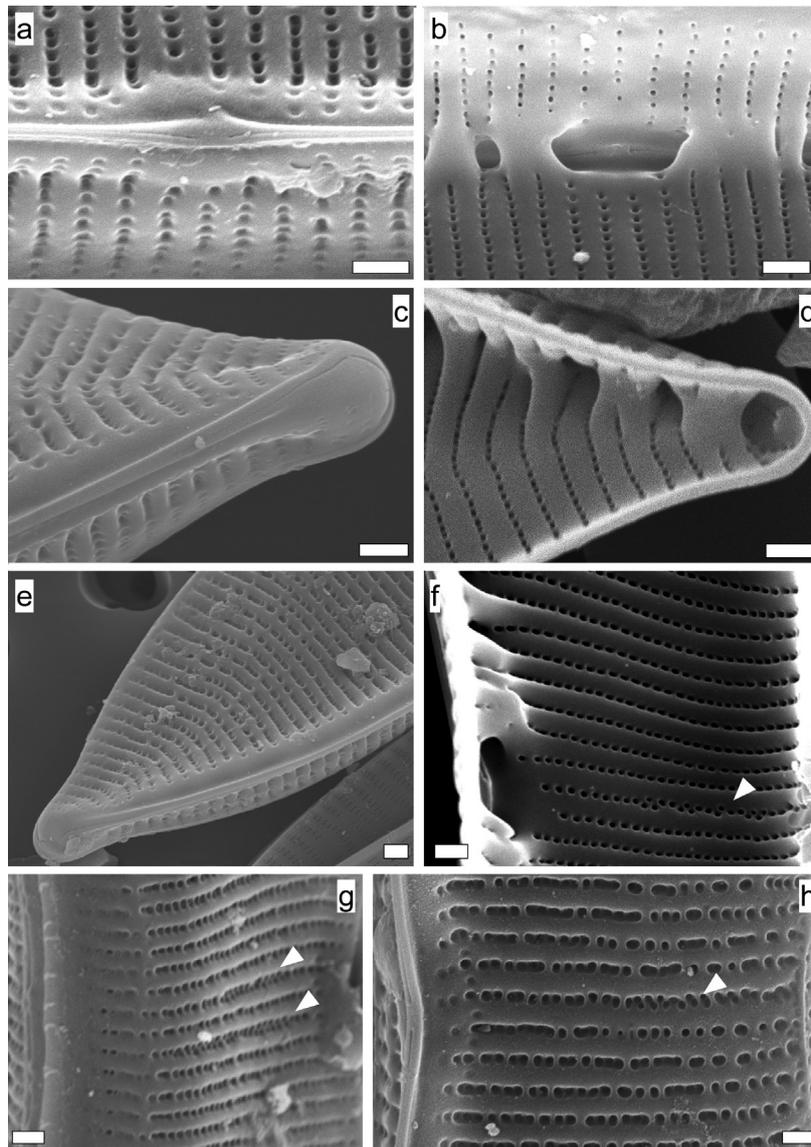


Fig. 6. SEM images of microstructures in *Nitzschia taikiensis* – raphe and areolae

Explanations: a – central raphe endings (external view), b – central raphe endings and large interspace in the central area (internal view), c and e – raphe at the apical end (external view), d – raphe at the apical end (internal view), f – double areolae around the central area (internal view) (arrow head), g-h – double areolae around the central area (internal view) (arrow heads), scale bar = 1 μ m

\equiv *N. amphioxoides* Hustedt 1959. Österr. Akad. Wiss. Math. Naturw., Kl. Abt. 1, 168: pl. 433. f. 21. 1959. (later synonym)

non *N. amphioxoides* Hustedt Explor. Parc Natl. Albrt, Mission H. Damas, 8:140. pl. xiii, f. 65-72. 1949. Holotype: plate 433, f. 21 in Hustedt (1959a)

Type slide: Hustedt No. W410 Salzlackengebietet, Burgenland, Austria.

Original type material: *Nitzschia amphioxoides* E9790, Szent Peter Ungarn.

Type locality: Salzlackengebietet, Burgenland, Austria

Description: Frustules rectangular in girdle view with rounded corners. Girdle usually composed of 7 bands (in SEM, 7-12 bands, Fig. 7). Girdle bands are ornamented with a single row of small pores, the open

ends of the bands occur at alternate ends of the frustule. Valves linear and slightly panduriform, with canal raphe constricted slightly in the middle and narrowly rostrate to capitate ends, 75-100 μ m long, 6-7 μ m wide. Canal raphe strongly eccentric, transapical striae easily resolvable under LM, ca. 20 in 10 μ m clearly punctuated, not interrupted by a longitudinal fold or narrowing. Fibulae 9-12 in 10 μ m, the two middle ones a little further apart from each other.

Comment: our LM and SEM measurements revealed that the specimens of *N. subamphioxoides* were 70-100 μ m long, 6-8 μ m wide, with 20-22 in 10 μ m transapical striae, and 5-8 in 10 μ m fibulae (n=50) (Fig. 8). The striae were arranged in parallel and from the valve center to the valve edge. However, they were slightly curved near the edge. One or two striae occurred

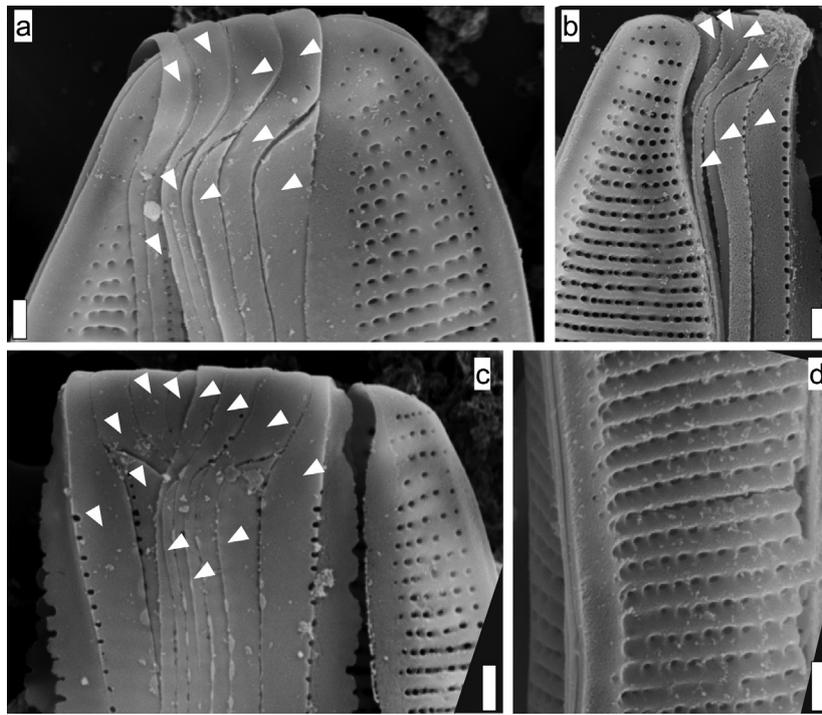


Fig. 7. SEM images of microstructures in *Nitzschia subamphioxoides* – bands and areolae

Explanations: a – girdle polar view showing 9 bands (arrow heads), b – oblique polar view showing 7 bands (arrow heads), c – girdle polar view showing 12 bands (arrow heads), d – external view of the central area showing areolae, scale bar = 1 µm

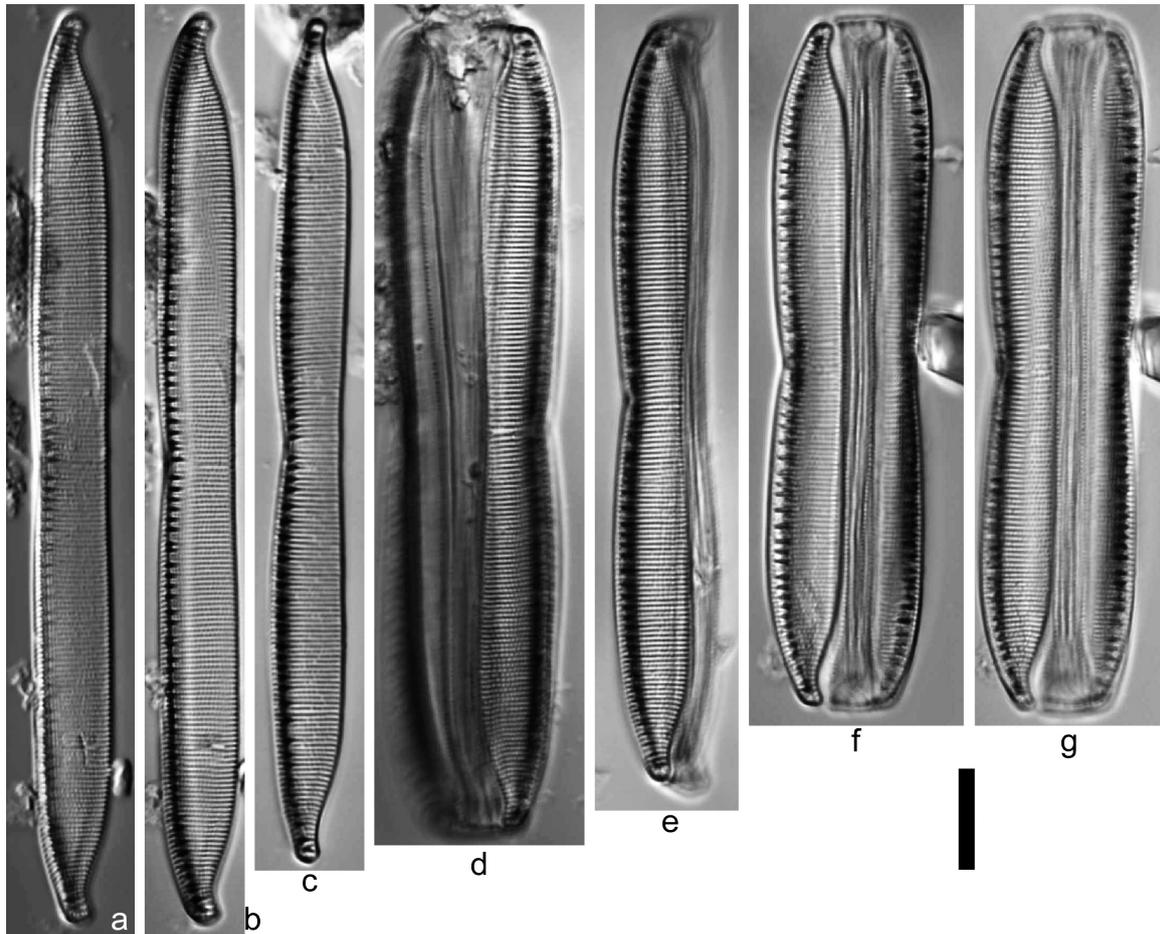


Fig. 8. LM images of *Nitzschia subamphioxoides* in the type material

Explanations: a-c and e – images valve views, d and f-g – girdle views, a-b and f-g – images of the same individual, scale bar = 10 µm

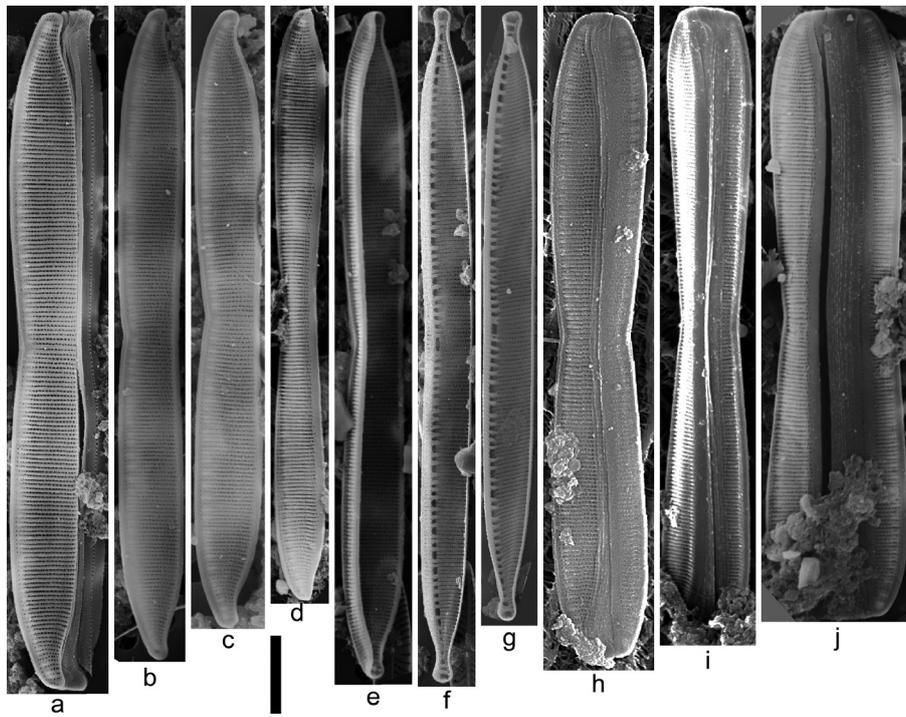


Fig. 9. SEM images of *Nitzschia subamphioxoides* in the type material
 Explanations: a-d – external views, e-g – internal views, h-j – girdle views, scale bar = 10 µm

between two fibulae and the spacing was heterogeneous. The central nodule was clearly recognised at one side of the valve edge.

SEM analysis: SEM images are illustrated in Figs. 7, 9-10. External valve surface only slightly convex, becoming flat towards margins, internal valve surface flat. The raphe sternum is elevated above the canal raphe surface. Canal raphe ornamented with a single row of areolae, except the valve middle where the areolae are missing (Fig. 10e, g). Raphe external and internal proximal ends (central nodule) present with external proximal ends simple and close to each other and internal proximal ends somewhat distant from each other (Fig. 10a-b). Distal raphe ends terminate in strong hooks bent to the same side, whereas internally form a distinct helictoglossae (Fig. 10c-d). Transapical striae composed of transapically elongated areolae, both internally and apically, 24-38/10 µm (mean: 30/10 µm). Rarely biseriate striae were rarely recognised in the

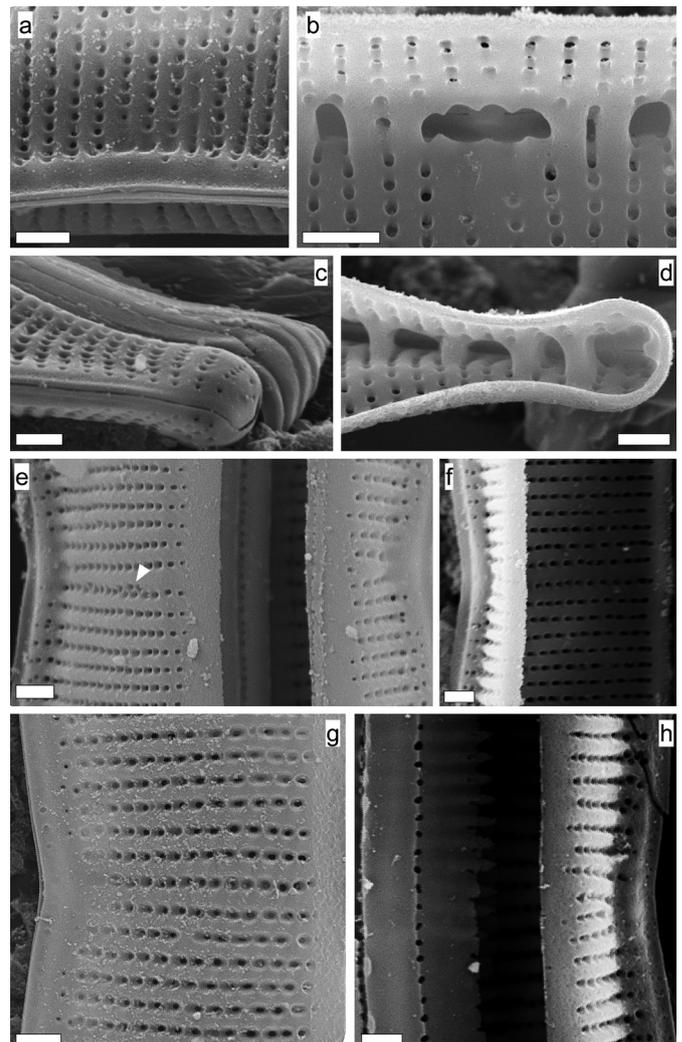


Fig. 10. SEM images of microstructures in *Nitzschia subamphioxoides* – raphe and areolae

Explanations: a – central raphe endings (external view), b – central raphe endings and large interspace in the central area (internal view), c – raphe at the apical end (external view), d – raphe at the apical end (internal view), e – double areolae around the central area (internal view) (arrow head), f – double areolae around the central area (internal view), g – single areolae around the central area (internal view), h – single areolae around the central area (internal view), scale bar = 1 µm

valve middle (Fig. 10). Fibulae massive, plain more or less of the same size, 5-8 in 10 μm .

Ecology: This species was discovered in a very low salinity and alkaline environment.

5. Discussion

5.1. Morphological features of *Nitzschia subamphioxoides* Hustedt

Hustedt (1959a) presented descriptions of *N. subamphioxoides*, except for the areola density, based on light microscopy analysis. On the other hand, the results of this study analysed the areola density and indicated that the frustules were more diverse in *N. subamphioxoides* compared to the description of the holotype (Figs. 7-10). The valve face was often flat. In the parts with a high areola density, that areolae sometimes seemed to be connected in LM images; however, the SEM images showed that they were separated. The actual areola density was, therefore, higher when observed under SEM than under LM (Hustedt 1959a). Although the length decreased with cell division, the width remained almost unchanged. The central nodule was clearly recognized both under LM and SEM. In addition, every parameter of frustules had a wider range than the corresponding parameter of *N. subamphioxoides* reported by Hustedt

(1959a) (Table 2). The results of the present study showed that morphological features such as the length, width, stria density, and areola density, were mostly consistent with those of *N. commutata*. Therefore, it is highly possible that *N. subamphioxoides* is the same species as *N. commutata*, as was suggested by Krammer & Lange-Bertalot (1988), Witkowski *et al.* (2000) and Hamsher *et al.* (2016).

5.2. Comparison between *Nitzschia taikiensis* and other *Nitzschia* species

Nitzschia taikiensis is morphologically similar to *N. subamphioxoides*, *N. commutata* [referred to as *N. piscinarum* Hustedt in Hustedt (1959a)], and *N. heufferiana* Grunow, but with differences in the keel and areola morphology (Hustedt 1959a) (Table 2). *Nitzschia taikiensis* also resembles *N. commutatoides* Lange-Bertalot in the LM images (Krammer and Lange-Bertalot 1988; Witkowski *et al.* 2000; Takano *et al.* 2009). However, specimens of *N. taikiensis*, had a higher mean length and coarser striae and areolae than specimens of *N. commutata* and *N. commutatoides* (Table 2). In addition, *N. taikiensis* had coarser striae and areolae than *N. heufferiana* (Table 2).

Multiple areolae that looked connected in the LM images, in the SEM images were clearly separated (Figs. 3, 6, 8). This characteristics distinguished them

Table 2. Comparison of characteristic features and environmental requirements of different *Nitzschia* species discussed in the text

Species	<i>N. taikiensis</i>	<i>N. subamphioxoides</i> (as <i>N. amphioxoides</i>)	<i>N. commutata</i>	<i>N. commutatoides</i>	<i>N. heufferiana</i>	<i>N. australocommutata</i>
Reference	This study	Hustedt (1959a)	Krammer & Lange-Bertalot (1988)	Krammer & Lange-Bertalot (1988)	Krammer & Lange-Bertalot (1988)	Hamsher <i>et al.</i> (2016)
Length (μm)	70-160	75-100 (*minimum: 70)	45-100	45-95	70-190	55-70
Width (μm)	7-13	6-7 (*maximum: 9)	5-8	7-12	4-7	5-6
Striae (/10 μm)	15-20	20 (*maximum: 22)	(18)19-23(24)	19-22	20-24(26)	20-21
Areolae (/10 μm)	SEM: 29-48	*SEM: 24-38	25	-	-	32-35
Fibulae (/10 μm)	5-9	5-6 (*maximum: 10)	9-12	6-10	14	8-10
Environment	Salt marsh	Low salinity lake	Coastal area	Tidal flat, Coastal lake	Freshwater lake	Freshwater lake
Salinity	Brackish	Brackish	Brackish	Brackish	Freshwater	Freshwater
pH requirements	Acidophilous	Alkaliphilous	**Alkaliphilous?	**Alkaliphilous?	Alkaliphilous (Lowe 1974)	Alkaliphilous (Hamsher <i>et al.</i> 2016)

Explanations: * – results of this study, ** – in the study of van Dam *et al.* (1994), the data on pH requirements of *Nitzschia commutata* and *N. commutatoides* are missing. Previous reports do not exclude the possibility that these species may be alkaliphilous (Hustedt 1959a; Krammer & Lange-Bertalot 1988; Veres *et al.* 1995; Krivograd Klemenčič *et al.* 2007; Baruah & Kakati 2012)

from *N. commutata* (Krammer & Lange-Bertalot 1988), however, made them similar to *N. subamphioxoides*. Viminous of *N. taikiensis* were irregularly spaced and their external openings sometimes merged with each other. These structural features were relatively similar to *N. commutatooides*. However, the specimen length and stria density differed considerably from those in *N. commutatooides*, i.e., in *N. taikiensis*, the length of specimens was higher and stria density lower compared to *N. commutatooides*. Due to the similarities in morphological characteristics, *N. taikiensis* is likely to be confused with *N. commutatooides*. Therefore, it is necessary to further study this species in detail and to accurately examine its occurrence and distribution. Genkal and Yarushina (2016) published the SEM images of *N. commutatooides* from the Naduiyakh River Basin (Yamal Peninsula, Russia), however, the morphological details of *N. commutatooides* were not clear in the images, therefore, it was difficult to compare the SEM images of *N. taikiensis* and *N. commutatooides*. However, the stria density of *N. taikiensis* was clearly higher than in the type material of *N. commutatooides*. On the other hand, Takano *et al.* (2009) published the LM images of *N. commutatooides* occurring around the Hachiro-gata lagoon in the Akita prefecture, Japan, which showed a lower density of striae. It is possible that the species reported by Takano *et al.* (2009) was *N. taikiensis*, because it had the same morphological features as *N. taikiensis*.

Nitzschia taikiensis was found more frequently in the lower salinity and lower pH environments in our study area (Table 1). The bottom sediment was peat, and there was almost no water flowing. Site 2 (salinity = 2‰, pH = 5.23) had the highest relative abundance of living and dead cells (Table 1). Our findings indicate that this species multiplies in an extremely low salinity environment, such as the upper limit of the tidal range. The salinity adaptation range of *N. taikiensis* is similar to that of *N. subamphioxoides* (Hustedt 1959a). However, *N. subamphioxoides* is reportedly an alkaliphilous species (Hustedt 1959a), and the frequency distributions presented in this study indicate that *N. taikiensis* is an acidophilous species (Table 1).

Herein, we present the first report showing SEM images and morphological features of both *N. sub-*

amphioxoides, in the type material, and a new species – *N. taikiensis*. *N. taikiensis* has not been previously reported in Japan, because most freshwater and salt marsh environments of Japan, excluding Hokkaido, have declined or been lost as a result of urban and industrial development (Sawai 2014). Additional information on this species and its habitat will be useful for future studies involving the reconstruction of paleo-environmental changes, such as sea level changes in coastal areas, based on diatom analysis.

6. Conclusion

We found *Nitzschia taikiensis* sp. nov. in the Toberi River marsh, eastern Hokkaido, Japan, and described this diatom as a new species. The sampling sites of *N. taikiensis* had very low salinity and pH levels, and were peaty. For comparison with this new species, we also provided the SEM images of *N. subamphioxoides*, because such SEM images had not been previously published. Identification of *N. taikiensis* was relatively easy based on the LM and SEM analyses of frustule features, such as the external form and stria density.

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