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Nitzschia arasensis sp. nov. (Bacillariophyta), a new diatom species from the Aras River and Dam, northwestern Iran

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Abstract
During recent investigations on the diatom and algal flora from the Aras Dam, northwestern Iran, an interesting epilithic diatom species belonging to the genus *Nitzschia* was collected and examined using both light and scanning electron microscopy. This diatom species shares morphological and taxonomic features with *Nitzschia clausii, N. kurzeana, N. filiformis, N. obtusa*, *N. lowei*, and *N. potapovae*; however, the taxon possesses narrow, elongate frustules that are weakly sigmoid in both valve and girdle views. The sigmoid character shows itself mostly at the apex of the valve and sometimes results in a rhomboid-like appearance; it is less clear in valve view and becomes weaker in the largest frustules, which are nearly symmetrical along the apical, transapical, and pervalvar axes. Valve ends are acutely rounded and blunt, but in shorter and more sigmoid valves, they become pointed to nearly triangular. Morphology and comparison with related taxa suggests the Aras Dam species is new and here described as *Nitzschia arasensis* Atazadeh, Ghavam *et* Edlund *sp. nov.* Water chemistry in the Aras Dam suggests that *N. arasensis* inhabits fresh to slightly brackish water.

Keywords
Bacillariophyceae; Brackish; Ecology; Morphology; New species; Taxonomy

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Introduction

The genus *Nitzschia* was described by Hassall (1845). *Nitzschia sigmoidea* (Nitzsch) W. Smith (1853; p. 38, pl. 13, fig. 104) is the type species for the genus; it substitutes for *Nitzschia elongata* Hassall (1845; p. 435, pl. 102, fig. 12), a later heterotypic synonym of *N. sigmoidea*. *Nitzschia* belongs to the class Bacillariophyceae, order Bacillariales and family Bacillariaceae (Spaulding & Edlund, 2008). *Nitzschia* species are characterized by the raphe located on the opposite sides of the two valves within each frustule; the raphe is eccentric and enclosed within a canal that passes through a marginal keel. The keel has internal support structures called fibulae, and within the broad *Nitzschia* complex, species also vary widely in size and shape. Valve lengths vary from a few to a few hundred micrometers. Striae extend transapically across the valve face and exhibit a wide range in density and arrangement as well (Krammer & Lange-Bertalot, 1988; Spaulding & Edlund, 2008).

Other important and diagnostic characteristics at the species level include state and shape of the proximal and distal endings of the raphe, fibulae and striae density in 10 µm, valve end shape, valve margin contours, and sometimes girdle view details. Taxonomy of the group can be very challenging (Trobajo *et al*., 2013), and the genus has been identified as non-monophyletic (Mann *et al.*, 2021). Nearly 3,000 *Nitzschia* species have been described (Kociolek *et al.*, 2020; Guiry & Guiry, 2021), and several

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groups have been segregated into separate genera, e.g., *Pseudo-nitzschia* H. Peragallo. *Nitzschia* species inhabit diverse freshwater to fully brackish and marine waterbodies and cross temperature, biogeochemical, moisture, and altitudinal gradients (Maja *et al.*, 2020), and their ecological characteristics have strong indicator value. For example, *Nitzschia* species are well-known for their adaptation to ecosystems with fine sediments and/or polluted and organically enriched habitats (Kociolek *et al.*, 2015; Lowe, 1974; Solak *et al.*, 2012). *Nitzschia* species may establish large communities hundreds of micrometers below the sediment surface and migrate diurnally or with tidal cycles to gather on the mud surface, creating a golden-brown film (Kociolek *et al.*, 2015).

We provide a taxonomic and morphological analysis of several populations of *Nitzschia* collected from aquatic habitats in northwestern Iran. Morphological data suggest that these collections contain a new species, which is described here as *Nitzschia arasensis* Atazadeh, Ghavam *et* Edlund.

Material and Methods

Benthic diatom samples were collected from rocky substrates from Aras Dam in northwestern Iran (Fig. 1) using standard methods (Stevenson & Bahls, 2006). Physical and chemical characteristics of the water, including temperature, pH, specific conductance, and dissolved oxygen were measured *in situ* using a Horiba multimeter (Water Checker). Other parameters, including total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS), were measured in the laboratory using a Hach DR 2800 spectrophotometer.

Diatom samples were digested in 35% H_2O_2 at 90 °C on a hotplate for 2 h, after which two drops of 35% HCl were added. Samples were filled with distilled water and left to settle overnight. The supernatant was discarded, and this rinsing/settling process was repeated at least four times (Battarbee *et al.*, 2002). Permanent slides were prepared using Naphrax mounting medium. Diatoms were identified using a Zeiss Axioplan 2 microscope with a plan Apochromat 100× oil-immersion objective (nominal numerical aperture: 1.3), using differential interference contrast (DIC). Images were captured with an Omax 35 digital camera. For scanning electron microscopy (SEM), rinsed samples were re-suspended in a solution of DI water for 30 min and then rinsed three more times with distilled water. Suspensions were dried directly on 22 mm diameter aluminum stubs and gold-coated with a Dynavac Xenosput sputter coater. Frustules were imaged in a TESCAN field-emission scanning electron microscope with a working voltage of 2.0 kV and spot size of 2. All algal samples from the Aras River used for ecological and floristic survey were archived in the ecology laboratory of the University of Tabriz (Iran). The terminology for valve and frustule features follows Round *et al.* (1990).

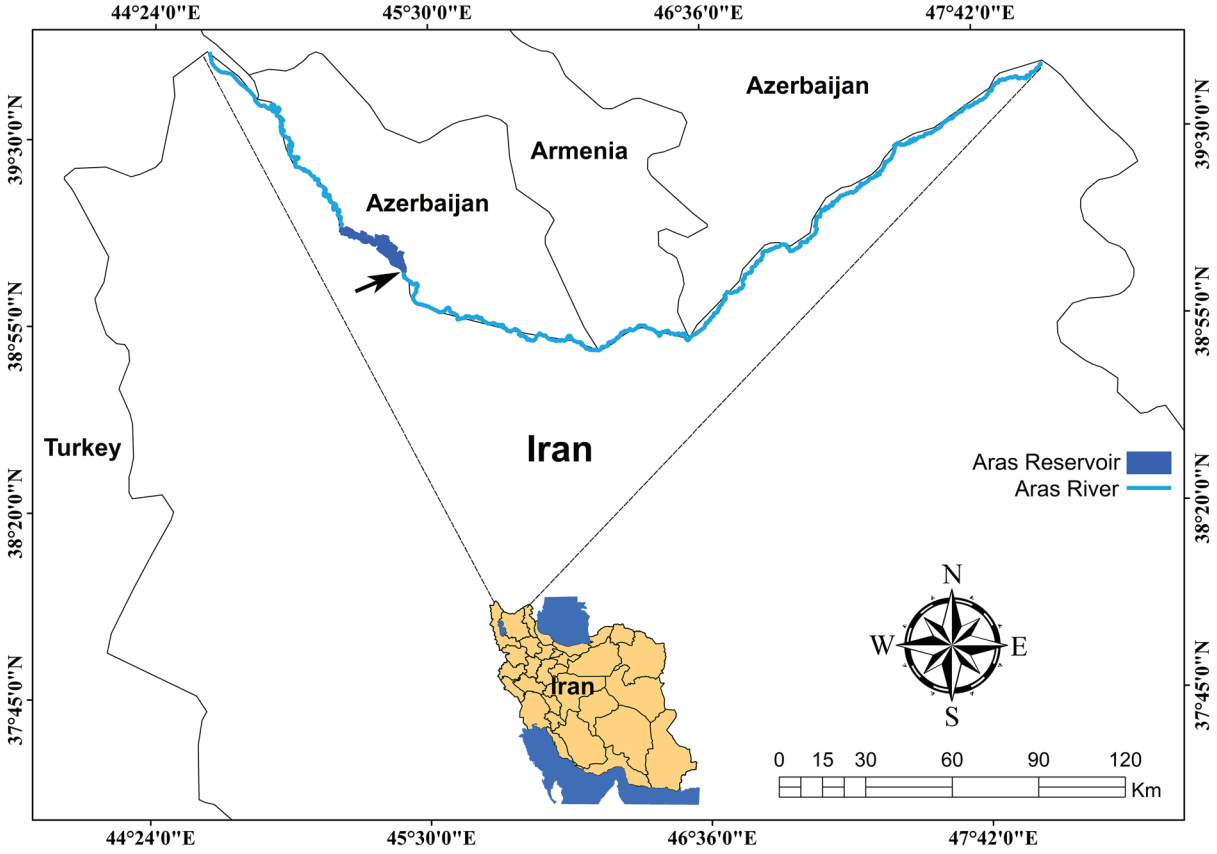


Fig. 1. Map of the Aras Dam and Aras River region in northwestern Iran.

Results

***Nitzschia arasensis* Atazadeh, Ghavam *et* Edlund *sp. nov*. (Figs 2–32)**

Description

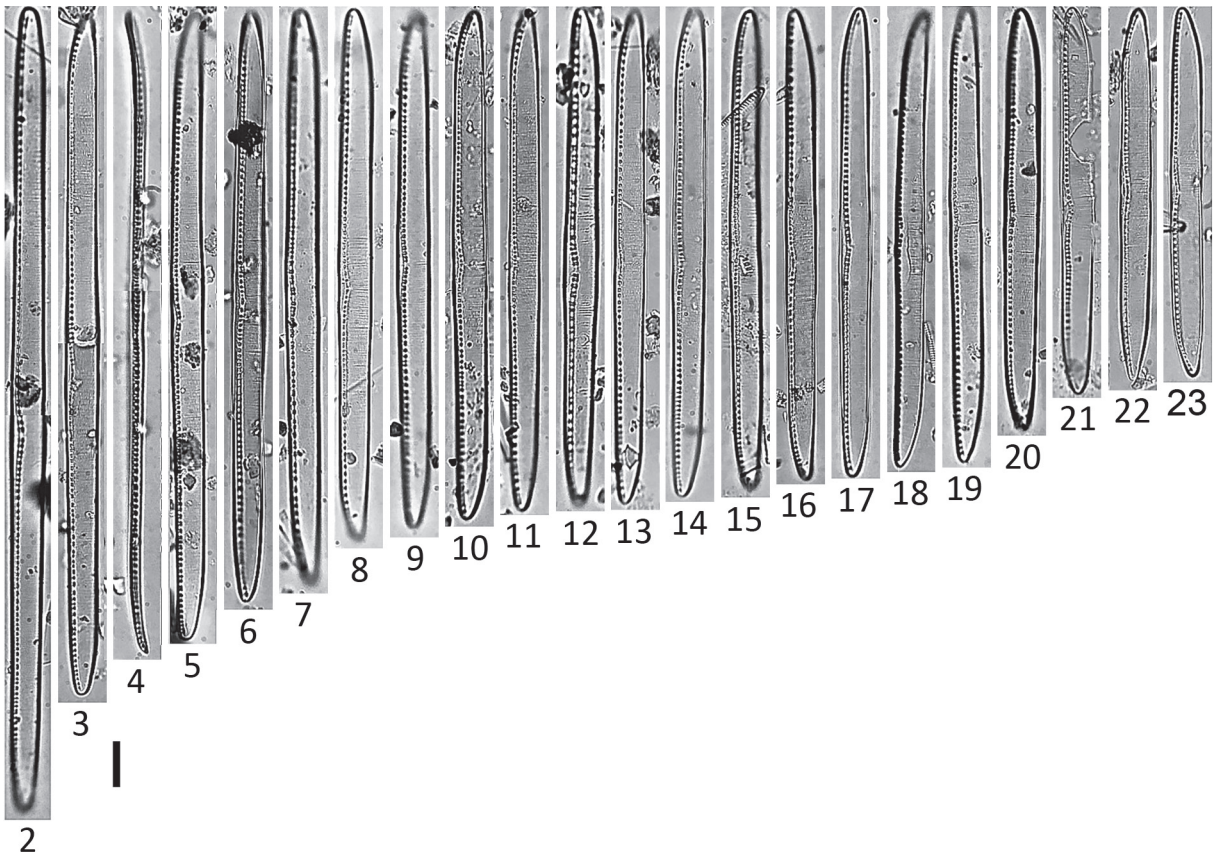
LM (Figs 2–23): Frustules narrow, elongate, and somewhat sigmoid in both valve and girdle view (Figs 2–23). The sigmoid character shows itself especially close to the apex of the valve (Fig. 18) and sometimes results in rhomboid-like appearance (Fig. 23); the sigmoid character is less clear in valve view compared to girdle view, and gets weaker in the largest frustules, which are nearly symmetrical along the apical, transapical, and pervalvar axes (Figs 2–3). Valve ends acutely rounded and blunt, but in shorter and more sigmoid valves, ends become pointed to nearly triangular (e.g., Figs 18, 23). Valve margins generally straight or slightly inflated near the valve center in larger valves (Figs. 3, 12). Valve length 90 to 223 µm (n = 180, mean 146.2±24.4 µm), valve breadth 7–10 µm (n = 180, mean 8.5±1.1). Striae fine, 29–31 per 10 µm and not always visible with light microscopy. Striae parallel throughout the valve, becoming slightly radiate towards the apices.

SEM (Figs 24–32): Raphe strongly eccentric (Figs 24–29), with ends visible only with SEM (Figs 27–28). External proximal ends of the raphe strongly curved inward and converge with the most central striae in SEM; internal proximal raphe ends meet in a thickened intermissio (Fig. 32). External distal raphe ends recurve completely towards the keeled margin and form a hook shape (Figs 27, 29). Internally, each distal raphe ends in a small helictoglossa (Figs 24, 26, 30). Externally, the keel bends strongly inward in the central area to form a shallow V-shaped area extending about ⅓ of the way across the valve face (Fig. 28). Keel internally supported by strong fibulae, typically 1–2 times broader than fenestrae (Figs 31, 32). Keel with 7–9 fibulae per 10 µm; fibulae internally separated by 1–2 interstriae (Figs 30, 31). Striae composed of uniseriate round to transpically elongate areolae, with the second areola from the raphe especially elongated externally. Areolae externally unoccluded and internally occluded by foricula (Fig. 32). Cingulum and chloroplast structure unknown.

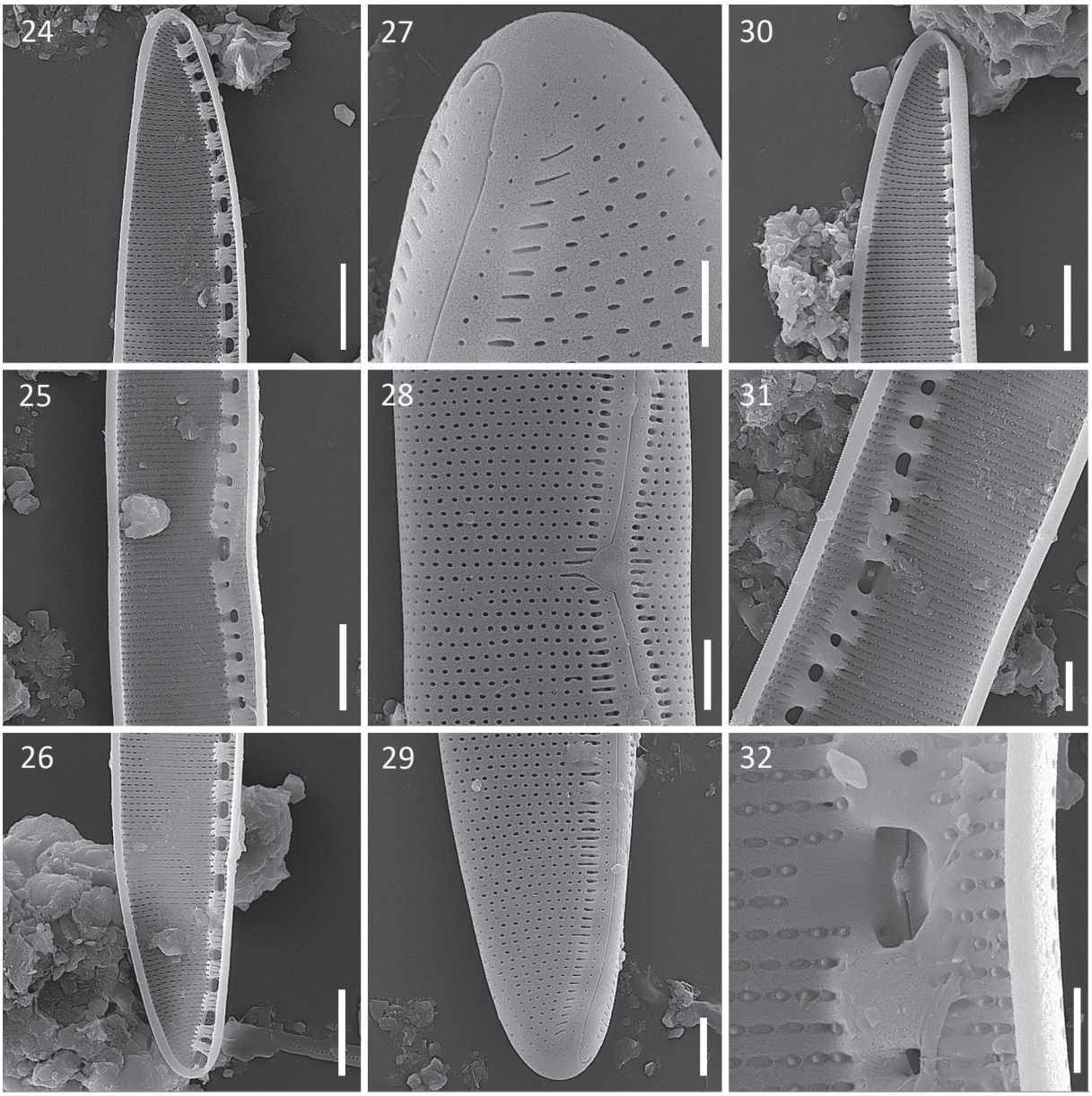
Holotype: Circled specimen on slide (Fig. 3, Meise Botanic Garden, BR-4920).

Isotypes: Circled specimen on slide (Fig. 10, Botanischer Garten und Botanisches Museum, Freie Universität Berlin, B 40-0045810). Circled specimen on slide (Fig. 12, Science Museum of Minnesota, SMM MBE-2605).

Type locality: Iran, Aras Dam, 39°08'30.2" N, 45°20' 41.5" E. Epilithic samples, collected by E. Atazadeh,



Figs 2–23. Light micrographs of size series of *Nitzschia arasensis sp. nov.*, Aras River and Dam, Iran. **(3)** Holotype. **(4)** Girdle view. **(10–12)** isotype. **(13–15)** Paratype. Scale bar 10 µm (2–23).



Figs 24–32. Scanning electron micrographs of *Nitzschia arasensis sp. nov.* **(24–26)** Internal valve view including fibulae, central area, and valve ends. **(27)** External distal raphe end is strongly recurved. **(28)** External proximal raphe ends deflect strongly dorsally, axial area with an adjacent row of very small areolae and a second row of larger, transapically expanded areolae. **(29)** External valve end. **(30)** Internal valve end with distal raphe ending in small helictoglossum. **(31–32)** Internal view of central area including fibulae and proximal raphe emerging from the canal with ends meeting in an intermissio. Scale bars 5 µm (24–26, 28–30) and 2 µm (27, 31, 32).

G. Dehghan, Z. Shojaei, and L. Sadeghi, 15 September 2019 (Meise Botanic Garden, material BR-4920).

Habitat: Epilithon.

Paratype: Circled specimen on slide (Fig. 15, Meise Botanic Garden, BR-4921).

Paratype locality: Iran, Aras Dam, 39°03'17.3" N, 45°26' 53.1" E. Epilithic samples, collected by E. Atazadeh, G. Dehghan, Z. Shojaei, and L. Sadeghi, on 15 September 2019 (Meise Botanic Garden, material BR-4921).

Etymology: The specific epithet, *arasensis*, refers to the type locality, the Aras River.

Ecology: *Nitzschia arasensis sp. nov.* is considered a fresh- to slightly brackish-water diatom. Populations were more abundant near the edges of the Aras Dam; secondary channels of the Aras Dam hosted even larger populations. Physicochemical parameters (Table 1) where *N. arasensis* was collected included pH neutral to slightly alkaline (pH 6.9 to 9.12). Total phosphorus concentrations were high (0.7 to 1.5 $mg \cdot l^{-1}$) in all samples, whereas total nitrate was 4–12 $mg \cdot l^{-1}$, and dissolved oxygen was 7–14 $mg \cdot l^{-1}$. The lowest and highest water temperatures detected were 14 °C and 28 °C, respectively. Total suspended solids (TSS) varied from a minimum of 400 $mg \cdot l^{-1}$ and maximum of 700 $mg \cdot l^{-1}$; total dissolved solids (TDS) were between 0.02 $mg \cdot l^{-1}$ and 0.75 $mg \cdot l^{-1}$. Specific conductivity (EC) was 0.3–1.68 $mS \cdot cm^{-1}$, and

Table 1. Physiochemical analysis of water samples (N = 40) of the Aras Dam and tributaries collected between September 2019 and October 2020, including range (minimum – maximum), mean, and standard deviation values.

Measured parameters	Range (min. – max.)	Mean & SD	Unit
pH	6.9–9.12	8.2±1.2	–
PO_4	0.7–1.5	1.1±0.05	$mg \cdot l^{-1}$
NO_3	4–12	2±1	$mg \cdot l^{-1}$
DO	7–14	10.5±3	$mg \cdot l^{-1}$
Temperature	14–28	21±4	°C
TSS	400–700	550±130	$mg \cdot l^{-1}$
EC	0.3–1.68	0.99±0.04	$mS \cdot cm^{-1}$
TDS	0.02–0.75	0.385±0.01	$mg \cdot l^{-1}$
BOD	3.45–7.4	5.425±2.1	$mg \cdot l^{-1}$

BOD was 3.45–7.4 $mg \cdot l^{-1}$.

Considering the high phosphate, nitrate, and suspended solids content, and moderate oxygen concentration, water quality in our study areas of the Aras River drainage was somewhat impacted; however, field notes indicate that sites were not heavily affected by human factors, like construction, sewage, and agricultural activities. Higher electrical conductivity was noted in the sub-branches of the river, which also contain larger populations of *N. arasensis*. There were also halophilic trees (i.e., *Tamarix* spp.; Tamaricaceae, Caryophyllales) nearby, additional evidence that these tributaries and sub-branches are more saline than the main waterbody.

Ecologically, other diatom species found in association with *N. arasensis* include: *Bacillaria paxillifera* (O. F. Müller) T. Marsson, *Amphora ovalis* (Kützing) Kützing, *Navicula oblonga* (Kützing) Kützing, *Tabularia fasciculata* (C. Agardh) D. M. Williams *et* Round, *Encyonema leibleinii* (Agardh) Silva *et al.*, *Hantzschia amphioxys* (Ehrenberg) Grunow, *Cymbella helvetica* Østrup, *Cymbella tumida* (Brébisson) Van Heurck, *Nitzschia clausii* Hantzsch, and *Nitzschia vitrea* var. *salinarum* Grunow. These taxa are similarly representative of slightly brackish conditions and flowing waters (Heudre *et al*., 2021).

Discussion

We compared *Nitzschia arasensis sp. nov*. to morphologically similar species, including *N. clausii* Hantzsch, *N. kurzeana* Rabenh., *N. filiformis* (W. Smith) F. Schütt, *N. obtusa* W. Smith, *N. lowei* Hamsher, Kociolek, Spaulding *et* A. I. Saleh and *N. potapovae* Hamsher, Kociolek, Spaulding *et* A. I. Saleh from among *Nitzschia* section Obtusae (sensu Krammer & Lange-Bertalot, 1988). These comparisons include morphological characteristics visible under LM and SEM (Table 2). The size range of *Nitzschia clausii* (Kociolek, 2011a; Krammer & Lange-Bertalot, 1988) is 20–68 µm (length) by 3–5 µm (width), which is much smaller than *N. arasensis sp. nov.*; *Nitzschia clausii* also has a lower L:W ratio than *N. arasensis sp. nov.* Valves of *N. clausii* are more visually sigmoid, even in larger specimens, and can have capitate and visibly curved ends. Proximal raphe ends bend sharply inward in *N. clausii*, but unlike *N. arasensis*, they extend to the valve center or beyond. The gap between the keel and proximal valve margin is not limited to the central area and may distinctly extend towards either end. Moreover, the fibulae density of 10–13 in 10 µm is more dense than that of *N. arasensis*.

In comparison to *Nitzschia arasensis, N. kurzeana* (Solak *et al*., 2012; Spaulding, 2010) has smaller valves (67–95 µm length and 6.5–8.5 µm breadth), more undulate margins, and a more distinct sigmoid appearance. Valve ends are wider than *N. arasensis*, and the distal ends of the raphe similarly form a wide hook-like shape, but in the opposite direction to *N. arasensis*. Fibulae density in *N. kurzeana* is 6.5–8 in 10 µm and similar to *N. arasensis*.

Compared to *Nitzschia arasensis, N. filiformis* (W. Sm.) F. Schütt (Kociolek, 2011b; Krammer & Lange-Bertalot, 1988) has more spindle-shaped frustules, more acutely pointed valve ends, and more symmetric valve shape. Length and width ranges are 27–150 µm and 4–5 µm, respectively, in *N. filiformis*, and striae are nearly as dense as *N. arasensis*, ranging from 29–34 in 10 µm, but appear more visible in light micrographs. The number of fibulae is similar in both species, at 7–9 per 10 µm.

Nitzschia obtusa W. Smith (Krammer & Lange-Bertalot, 1988) is morphologically closest to *N. arasensis* among these comparable species. Among its numerous varieties, *N. obtusa* has a broad range of morphological parameters; however, *N. obtusa* is the only taxon among the six comparable species that is similar size to *N. arasensis*. The length of *N. obtusa* is 120–350 µm, and the breadth range is 7–13 µm. Striae density of *N. obtusa*

Table 2. Morphological comparison of *Nitzschia arasensis sp. nov.* with *N. clausii*, *N. kurzeana*, *N. filiformis*, *N. obtusa*, *N. lowei*, and *N. potapovae*.

Taxon	Length range (µm)	Width range (µm)	Striae density (#/10 µm)	Fibulae density (#/10 µm)	Overall frustule shape	Proximal raphe ends	References
N. arasensis sp. nov.	90–223	7–10	29–31	7–9	Linear-elongate; weak sigmoid shape	Proximal ends bend ventrally	this study
N. clausii	20–68	3–5	NA	10–13	Linear, slightly concave at the middle	Proximal ends bend ventrally	Kociolek, 2011a; Krammer & Lange-Bertalot, 1988
N. kurzeana	67–95	6.5–8.5	25–40*	6.5–8	Linear elongate with slight to moderate sigmoid shape	Proximal ends bend ventrally	Solak *et al*., 2012; Spaulding, 2010
N. filiformis	27–150	4–5	29–33	7–9**	Linear to linear-lanceolate	NA	Kociolek, 2011b; Krammer & Lange-Bertalot, 1988
N. obtusa	120–350	7**–13	22–30	5–6	Rhombic or linear elongate with distinct sigmoid shape	NA	Krammer & Lange-Bertalot, 1988
N. lowei	119.6–182.8	6.6–9.8	NA	5–7	Valves elongate, with apiculate ends	NA	Hamsher *et al*., 2021
N. potapovae	182–346	7–10**	20–22	5–6	Valves sigmoid, linear and elongate with bluntly rounded apices	Strongly bent and extend onto the valve	Hamsher *et al*., 2021

*Estimated number, **Identical numbers to *N. arasensis sp. nov.* morphometrics

is 22–30 per 10 µm, which tends slightly coarser than *N. arasensis*. Fibulae are less dense too in *N. obtusa*, at 5–6 per 10 µm. In further contrast to *N. arasensis*, frustules of *N. obtusa* are generally more sigmoid and in smaller valves may even have a distinct rhomboid shape. Valve ends in *N. obtusa* range from broadly rounded to pointed or triangular or even slightly capitate with a narrowed neck. Undulation in valve margins is deeper than in *N. arasensis sp. nov.*, especially in the valve center. Some specimens of *N. obtusa* also have a slightly constricted margin at the valve center, which, based on our observed populations, is a characteristic absent in *N. arasensis*.

Comparison between *Nitzschia arasensis* and *Nitzschia lowei* (Hamsher *et al*., 2021) shows that *N. arasensis sp. nov.* has larger valves, which are elongate with a weak sigmoid shape, whereas *N. lowei* valves are elongate, with smaller apiculate ends. The length of *N. lowei* is 119.6–182.8 µm and the breadth range is 6.6–9.8 µm, which bears similarities to *N. arasensis*. Finally, comparison between the new taxon and *Nitzschia potapovae* (Hamsher *et al*., 2021) indicates that *N. potapovae* has linear to elongate valves with bluntly rounded ends. The length of *N. potapovae* is 182–346 µm, and the breadth range is 7–10 µm. Striae density of *N. potapovae* is 20–22 per 10 µm, which is much coarser than *N. arasensis*.

A number of biomonitoring and floristic studies have been performed over the past few decades to document diversity and ecology of Iranian algae including studies on the *Algues des dèserts d'Iran* (Compère, 1981), Lut Desert (Kheiri *et al*., 2024), Caspian Sea (Fallahi, 1991; Fatemi *et al*., 2005), Anzali Lagoon (Nejadsattari *et al*., 2005), Lake Neure (Nejadsattari, 2005), Gharasou River (Atazadeh *et al*., 2007), streams in Ramsar (Soltanpour-Gargari *et al*., 2011), Karaj River (Kheiri *et al*., 2018), Balikhli River (Panahy-Mirzahasnlou *et al*., 2018), Taleghan River (Naseri e*t al*., 2022), Western Rivers of Lake Urmia (Mehrjuyan & Atazadeh, 2022), and the Ahar Chai River (Yadollahi & Atazadeh, 2024).

However, there are still gaps in our knowledge of the diatom flora of Iran due to few floristic studies and the availability of taxonomic literature and resources. As a result, knowledge of the number of species, their biogeography, and the distribution of Iran's diatoms is still growing. This new knowledge is informing diatom metrics, which are important tools to assess the ecological/environmental condition of riverine ecosystems (Atazadeh *et al*., 2021). Indeed, diatoms respond directly to physical, chemical and biological changes in rivers and streams, because they are sensitive to many drivers of change in aquatic ecosystems (Kelly & Whitton, 1995; Lowe & Pan, 1996; Prygiel *et al*., 2002; Hill *et al*., 2000; Chessman *et al*., 2007; Oeding & Taffs, 2017; Atazadeh *et al*., 2021).

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