

SHORT COMMUNICATION

First record of the diatom *Nitzschia navis-varingica* in the Sea of Marmara

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ABSTRACT

We report the first occurrence of a diatom species, *Nitzschia navis-varingica* in the Sea of Marmara. Previously, this species was observed in the Mediterranean Sea in 2016. Samples were collected from the coast of Darica, Istanbul, Turkey, on 22 June 2021 during a large-scale mucilage event. The species was isolated, cultured under laboratory conditions, and investigated with scanning electron microscopy and light microscopy.

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Introduction

The diatom *Nitzschia navis-varingica* Lundholm & Moestrup was first identified in Vietnam waters in 1997 (Kotaki et al., 2000) and later observed in Japan and the Philippines (Kotaki et al., 2004), Australia and New Zealand (Chiovitti et al., 2005), Thailand (Romero et al., 2008) and Malaysia (Suriyanti & Usup, 2015). The first report of this species in an area outside the Western Pacific was from the Mediterranean Sea in 2016, when Ayaz et al. (2018) observed it off Erdemli

along the southern Turkish coast. This species is generally reported to dominate in benthic, brackish environments (Kotaki et al., 2004; Romero et al., 2012) at salinities ranging from 5 to 33 (Lundholm & Moestrup, 2000; Tan et al., 2016). However, the Mediterranean Sea strain (Strain no: MED_01, Accession number: MW315998) grows at higher salinities of 38 (Eker-Develi et al., 2020).

The purpose of the present study is to report for the first time the presence of the diatom *N. navis-varingica* in the Sea of Marmara.

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Material and Methods

By dipping a plastic container of 10-liter volume, a seawater sample was obtained on 22 June 2021 from the shore where mucilage accumulation was dense in the Sea of Marmara located at Darica, Istanbul (40.75 N, 29.39 E, Fig. 1). The temperature and salinity of the seawater were 23.2°C and 23.7, respectively (Hanna HI98319 salinity tester).

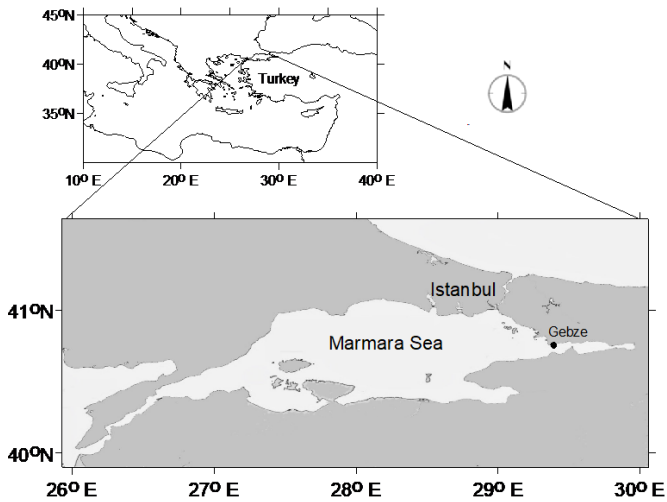


Figure 1. Sampling area for the *Nitzschia navis-varingica* on 22 June 2021.

In the laboratory, during the initial observation of the sample under the microscope, the phytoplankton species identified included one species of the dinoflagellate (i.e., *Prorocentrum micans*) and several species of diatoms (i.e., *Cylindrotheca closterium*, *Pleurosigma* sp., *Skeletonema* sp., *Licmophora* sp., *Dithylum brightweli* and several unidentified pennates). F/20 Medium was added to the seawater sample when cell numbers of the microalgae were observed to increase. *N. navis-varingica* cells were selected with thinned glass Pasteur pipettes. Cultures were grown under a 12h:12h light-dark cycle at 20°C temperature and ~20-30 $\mu\text{mol photons s}^{-1}$ irradiance in 100 ml Erlenmeyer flasks.

Results and Discussion

Cell sizes of 50 individuals were measured from a three-week old culture with a light microscope (Nikon Eclipse TS100) at 1000X magnification. For scanning electron microscope (SEM) images, samples were first washed with distilled water, acid cleaned, re-washed, and filtered through 0.2 μm cellulose acetate filters. Filters were coated with gold using a Quorum Q150R Sputter Coater and examined with a field emission scanning electron microscope (Quanta 650 Field Emission SEM).

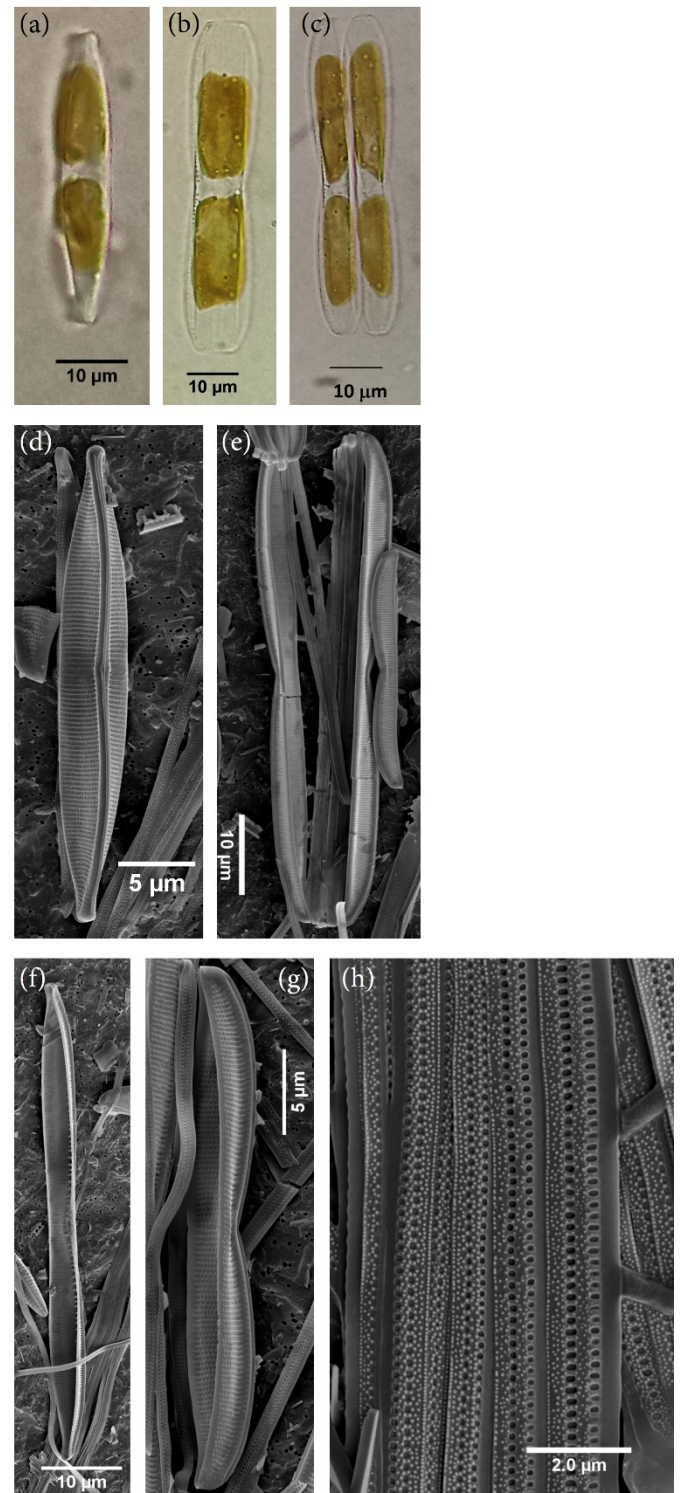


Figure 2. Light microscope (a, b, c) and SEM (d, e, f, g, h) images of *N. navis-varingica*. (a) valve view, (b) girdle view, (c) girdle view of doublet cells, (d) valve view showing the central nodule, (e) girdle view with open bands and two cells having different sizes, (f) internal view showing fibulae and interstriae, (g) girdle view with areolae, arrow shows an open band with two rows of areolae (h) cingular bands.

N. navis-varingica was observed for the first time in the Sea of Marmara in this study. Previously, it was recorded in the Mediterranean Sea in samples isolated in 2016 (Ayaz et al., 2018). However, this species must have been introduced to the Mediterranean Sea before 2011 since its picture was observed in a culture sample from 2011 (unpublished data of Eker-Develi). DNA sequence analysis may show the differences between the Mediterranean and the Marmara Sea strains.

From the world's oceans, the neurotoxin Domoic acid is known to be produced by only two species of approximately 900 species of *Nitzschia*, namely *N. bizertensis* Smida, Lundholm, Sakka and Mabrouk and *N. navis-varingica* (which is the subject of this paper) (Lundholm & Moestrup, 2000; Bouchouicha-Smida et al., 2015) besides 28 out of 58 *Pseudo-nitzschia* species (Bates et al., 2019; Dong et al., 2020; Chen et al., 2021; Olesen et al., 2021) as well as by the red alga *Chondria armata* (Bates et al., 2019).

Domoic acid production of *N. navis-varingica* in different regions has been demonstrated by several studies (Kotaki et al., 2000, 2004, 2005, 2008; Romero et al., 2012; Tan et al., 2016). While in most of these studies, *N. navis-varingica* is shown to produce domoic acid, some other investigations reported production of the closely related isodomoic acid A and/or isodomoic acid B (Bates et al., 2018). A few non-toxic strains have also been recorded from the Philippines (Kotaki et al., 2005) and Malaysia (Suriyanti & Usup, 2015). Domoic acid was not detected in the Mediterranean Sea strain, for which the presence of isodomoic acid was not screened (Eker-Develi et al., 2020). The toxicity of *N. navis-varingica* has been suggested to pass from parent to daughter strains (Romero et al., 2011; Bates et al., 2018).

We have not yet undertaken any study on its toxins; however, toxicity analysis will also elucidate its potential risks to the newly introduced regions. The Sea of Marmara is an essential area for mussel production, both from the capture fishery and mariculture (over 4 thousand tons from Turkish waters; <https://data.tuik.gov.tr/Bulten/Index?p=37252&dil=2>). Therefore, studying the toxicity of this species is necessary as there are also health implications concerning human consumption. The Sea of Marmara is already jeopardized by the large-scale mucilage events (Aktan et al., 2008; Tüfekçi et al., 2010; Balkıs et al., 2011; Balkıs-Ozdelice et al., 2021; Savun-Hekimoğlu & Gazioğlu, 2021) and mass occurrence of another toxic species may further aggravate ongoing environmental problems in this sensitive ecosystem. The toxicity of this species and its interaction with other dominant phytoplankton species

in the environment should be investigated to understand the potential relation of *N. navis-varingica* in such mucilage events.

The size range of this species from an isolate in a shrimp culture in Vietnam were 45-55 µm long and 9-11 µm wide in girdle view (n= ~30; Lundholm & Moestrup, 2000) which are narrower than our respective measurements of 21-56 µm and the 4-13 µm here for the Sea of Marmara isolate.

This species may have also been introduced to the vulnerable Black Sea ecosystem. Since this species is unknown by many investigators, it is possible that it was overlooked by many taxonomists or recorded only as a pennate diatom species.

Compliance With Ethical Standards

Authors' Contributions

EED: Analysis and Investigation, Methodology, Conceptualisation, Writing, Original draft, Review.
AEK: Sampling, Writing, Conceptualisation, Data curation, Validation, Review.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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