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RESEARCH PAPER

Exploration of *Nitzschia* from the Coastal Water of Suak Ribee, West Aceh Regency, Indonesia

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Abstract

Indonesia, a critical biodiversity hotspot, harbours diverse ecosystems. Diatoms, siliceous microalgae, are crucial in primary production and oxygen generation globally. With over 100,000 identified species, diatoms exhibit vast morphological diversity influenced by their unique cell division and life cycle. They are significant food sources for zooplankton, contributing to silica sequestration in aquatic environments and vital biological indicators of water quality, responding to environmental changes. The genus *Nitzschia*, a diverse group comprising nearly 3,000 names classified within the Sigmoideae group, has been found valuable in aquaculture due to its fatty acid content. This study isolates and identifies *Nitzschia*, a diatom from Suak Ribee Beach, West Aceh Regency, Indonesia, utilizing morphological characteristics for species identification. The research aims to contribute to understanding diatom diversity and ecological roles in this coastal environment.

Introduction

Indonesia is one of the most critical biodiversity hotspots in the world. A promising approach to identifying areas of high biodiversity is to identify 'hotspots' or areas determined by 'biological commonalities'. Each hotspot features a separate biota or community of species that fit together as a biogeographic unit (Myers et al., 2000). Diatoms are siliceous microalgae of high ecological importance (Hamsher et al., 2021). Diatoms (Bacillariophyta) represent a substantial source of primary production and oxygen locally and globally. The diatom group represents a highly diverse group of algae, with more than 100,000 species identified to date (Mann, 1999; Lewis, 2007). Due to the peculiarities of diatom cell

division and the life cycle, morphological diversity may frequently result from physiological differentiation. It has been estimated that they generate up to 25% to 100% of all oxygen on earth. Diatoms are a substantial food source for zooplankton and a significant component of silica sequestration in freshwater ecosystems and oceans (Minelli, 2016). They are the most effective primary producers of water in various ecosystems on earth, growing under a wide range of environmental conditions, and have become the subject of considerable attention in many countries due to their potential applications in various fields (Risjani et al., 2021). Diatoms can be utilized as biological indicators of the quality of aquatic environments (Sheath & Wehr, 2015). Diatoms in the Bacillariophyceae class demonstrate the capacity to alter their composition in response to changes in water quality, including alterations in quantity, abundance, and the presence of other taxa (Prasertsin et al., 2021).

The genus *Nitzschia*, with nearly 3,000 names, is one of the largest genera of diatoms (Kociolek, 2004). The genus *Nitzschia* A.H. Hassall 1845 is the most speciose genus within the diatom family Bacillariaceae Ehrenberg and in general is regarded as one of the most speciose among diatoms (Solak et al., 2012). *Nitzschia* species are highly resistant to lethal compounds, including organic pollutants and the most degraded industrial and municipal waters (Bates et al., 2018) most *Nitzschia* species inhabit benthic habitats (Round et al., 1990). The genus *Nitzschia* is classified within the group Sigmoideae (Hamsher et al., 2021). In addition, this pennate diatom serves an important primary role in aquatic ecosystems as a producer of phytoplankton, and as a live feed in aquaculture farms due to its high content of fatty acids (Shi et al., 2008; Pau et al., 2021).

Suak Ribee Beach is in West Aceh Regency, Aceh Province, Sumatra Island, Indonesia. It is identified as one of the beaches in the area characterized by dark-colored sand. The beach is located near residential areas, with minimal vegetation surrounding it. It is a popular tourist attraction. The most genera in this preliminary research belong to the class Bacillariophyceae. According to Fitriyah et al. (2016); Mirzaei et al. (2017), Bacillariophyceae has a high survival rate in surface-water environments. This is also interpreted by Arsad et al. (2021) as evidence that Bacillariophyceae have attachment devices, such as gelatinous stalks, which facilitate their attachment to the substrate and enable them to adapt to a range of current speeds, from 0.1 to 1 m/s.

The morphometric characteristics of frustules, which are necessary for such identification, are visible only by scanning or transmission microscopy (Arapov et al., 2017). This study aims to investigate the diatoms of the genus *Nitzschia* by isolating them from the waters of Suak Ribee Beach and identifying them based on their morphological characteristics.

Materials and methods

Time and sampling location

This study was collected in January 2023. The sampling point was located at Suak Ribee Beach, West Aceh Regency, Aceh, Indonesia with coordinates of 4°9'12.46"N and 96°6'31.82"E. The sampling in bottles was transported to the Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia. Isolation of samples was conducted at the Hydrobiology Laboratory, Department of Fish Resources, Universitas Brawijaya, and the morphological analysis using a scanning electron microscope was undertaken at the Minerals and Advanced Materials Laboratory, State University of Malang, East Java, Indonesia.

Sampling method

It is essential to select appropriate sampling sites that are proximal to discharges, accessible, and possess previous monitoring data. In this study's preliminary stage, the collection of samples from wild habitats is intended to explore the diversity of the area. Kelly et al. (2019), the sampling procedure was conducted by the standard methodology utilized in similar studies. Diatom sampling can be accomplished in a variety of ways, including the collection of samples on a substrate or sediment, by the use of a rattle to dislodge samples from rocks in the water (Solak et al., 2020), and by the sampling of water columns by using a plankton net mesh size 20 μm vertically and horizontally (Pane et. al., 2023). Additionally, samples were collected by brushing a surface area of 50-100 cm^2 of rock and sand sediment was transferred into the 50 mL bottles.

Figure 1. The sampling site is located in Suak Ribee, Aceh, Indonesia.

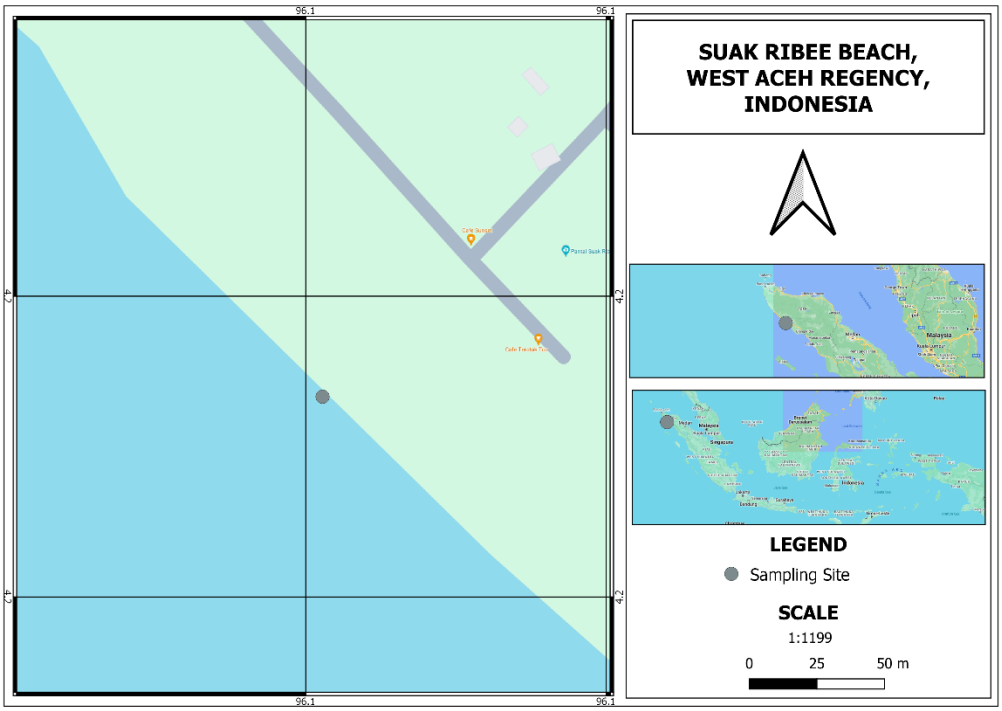

Figure 2. The mapping of the sampling locations for this study.

The initial activity was sterilization, which aimed to eliminate unwanted microorganisms (contaminants) from tools and containers used for diatom cultivation. The equipment, including test tubes, erlenmeyers, and aeration hoses, was soaked in a chlorine solution at a dose of 1 mL per 1 L of water. The solution was allowed to act for 24 hours, after which the equipment was rinsed with clean water and dried. The medium utilized in this research is seawater. From the isolation stage onwards, this medium was employed to maintain stable culture environment conditions. The medium was treated using the standard method or pure culture, achieved using F/2 Walne fertilizer and vitamin B12. The axenic culture begins with preparing erlenmeyer flasks, which were of the requisite size of 250 m and prepared in triplicate. The inoculants that have been prepared are then included in the erlenmeyer flasks. Accordingly, the pure cultures required development through the repetition of transfer steps. The optimal temperature for diatom culture was 24 °C, maintained by a fluorescent lamp at a distance of approximately 30 cm. Jiang et al. (2020), in order to ensure the continued cultivation of the diatom biomass, it was necessary to expand the scale of production. The initial diatom culture stage should be prepared in the log growth phase. The harvesting of diatoms should commence when they begun to detach from the substrate, typically around 6-7 days. Centrifugation of the culture should be

employed to collect the diatom biomass as a preservation method before SEM identification.

Identification

The observed characteristics were identified through morphological diversity. The text identifies and provides the referenced literature for each species' description. Secondary data on local and similar records were collected and compiled from previous studies and books. The morphological characteristics of the diatom isolate were then identified through descriptive analysis using the diatom identification books Davis (1955), Prescott (1970), Witkowski et al. (2000), and diatoms.org.

Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) was performed using a Merk FEI instrument. The Inspec-S50 is a device that allows for the inspection of materials at the nanoscale. The sample to be analyzed was positioned on a holder with a precision of ±10 mm. Samples were used for analysis using an Au-Pd coating to enhance conductivity. The sample was subsequently inserted into the SEM chamber and then subjected to vacuum pumping. Once the chamber was completely evacuated, the SEM machine was ready (beam on).

Results and discussion

Description

The genus *Nitzschia* has several species with different characteristics. These genera present variations in valve width, presence or absence of a central interspace, fibula and stria density, and other features that distinguish these species. Oliver et al. (2021), the genus *Nitzschia* includes a diverse range of species with varying cell sizes, with the majority falling within the range of 5–70 µm in length. Based on morphological identification, *Nitzschia*, a diatom species initially collected from coastal waters in Suak Ribee, West Aceh Regency, Aceh, Indonesia, was isolated in axenic culture by ALGAEn. The isolate is euryhaline and capable of cultivation in seawater and low-salinity brackish water, with the ability to grow using a CO_2 supply.

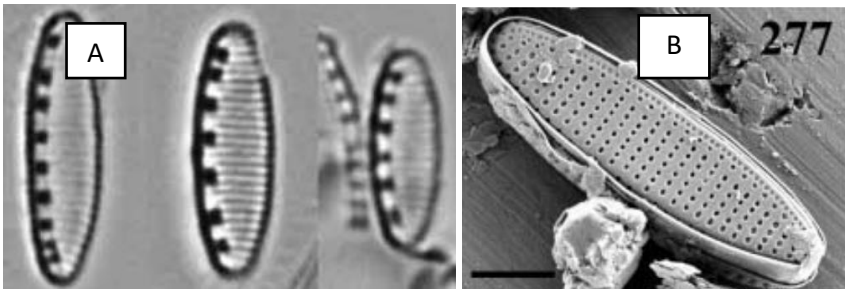

Figure 2. A. The micrograph of *Nitzschia soratensis*, obtained from the Sorata Department in Bolivia, ANSP GC 26804 (Trobajo et al., 2013); B. The SEM image of *N. soratensis* with the scale bars 2 and 5 µm (Morales and Vis, 2007).

Determining species based on cellular morphology can be challenging within the genus *Nitzschia* (Figure 4). The examined strain exhibits similarities to *Nitzschia soratensis* (Figure 3) (Morales and Vis, 2007; Trobajo et al., 2013; Hamsher et al., 2016; Puccinelli et al., 2019). The isolate exhibits larger valves that are linear or parallel sides, becoming elliptical in smaller specimens. The apices are broadly rounded. The frustules are rectangular in girdle view with the canal raphe and fibulae relatively large. Round et al. (1990), a fibulae is defined as an internal bar or silica strut that supports the raphe canal. In the plural form, the term "fibulae" describes these structures. The fibulae transapically extend from the valve face to the raphe canal. Genera known to contain fibulae examples include *Denticula*, *Nitzschia*, and *Surirella*. Striae are present in the centre of the valve and are arched near the poles, a feature that is particularly evident in smaller cells. Areolae are visually present in some larger specimens. Valve length 8.0 µm and valve width 3.0. In terms of length, the specimen in question measures 8,247 µm, while its width ranges from 2 to 3 µm (Figure 4).

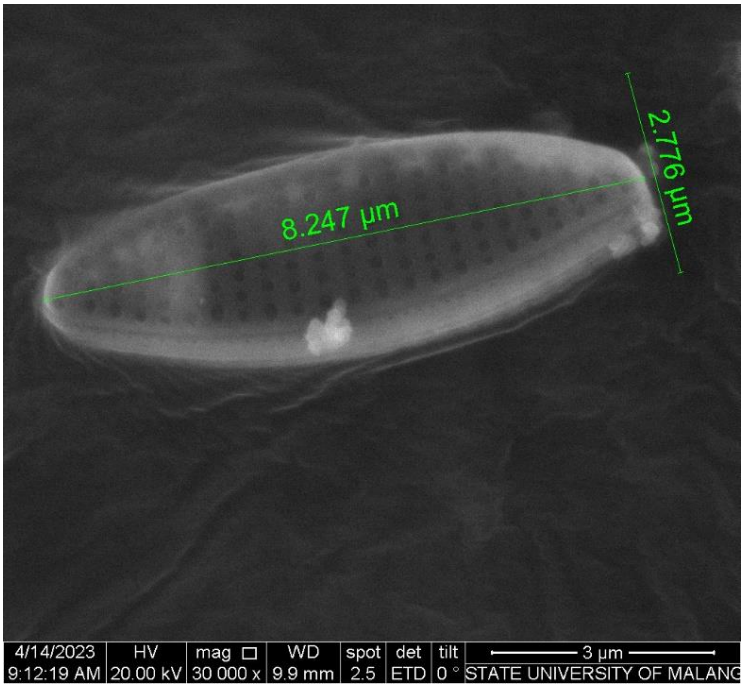

Figure 3. SEM of *Nitzschia* isolated from Suak Ribee Beach.

Nitzschia cells are typically elongated in shape, either linear or lanceolate, and *Nitzschia* species may exist as solitary cells or in colony form. Most *Nitzschia* species have two chloroplasts in each cell, one at the anterior end and one at the posterior end. Each valve has a raphe that is eccentrically positioned and supported by fibulae. The two raphe structures of a frustule are diagonally opposite, which is a defining characteristic of the genus (Nitzschioid) (Lundholm and Jvind Moestrup, 2000; Carballeira et al., 2017; Solak et al., 2021). The number of striae and fibulae on the valve can differ between *Nitzschia* species. One study reported 15 fibulae and 31 striae per 10 µm in a potentially new *Nitzschia* species (Louw et al., 2018). The identification of species to their respective taxonomic levels remains a challenging endeavor due to the vast array of species present.

Distribution

A number of species belonging to the genus *Nitzschia* are found in a wide variety of marine habitats, from the equatorial to the polar regions (Cipolletta et al., 2022). The vertical distribution of phytoplankton species is influenced by both environmental factors, such as the physical structure of the water column, irradiance, and nutrient availability, and biological characteristics of the species, including the presence of flagella, the capacity to regulate buoyancy, and adaptation to low light environments. Nevertheless, phytoplankton are capable of accumulating and even growing in subsurface chlorophyll maxima, and in correspondence with density gradients, where they can form thin phytoplankton layers (Durham and Stocker, 2012). The geographical distribution of certain species of Pseudo-nitzschia is limited to specific regions, which are classified as tropical, temperate, or cold-water species (Teng et al., 2012).

However, our isolate is considered a tropical species. It is very rare to study *Nitzschia* in this area, particularly in Suak Ribee, West Aceh Regency, Aceh, Indonesia. Risjani et al. (2021) identified the highest abundances of *Nitzschia frustulum* (6,8%) and *Nitzschia inconspicua* (4,9%) on the east coast of East Java. These were observed on the beaches of Tiga Warna, Watu Pecah, Clungup, and Gatra. Rachman and Thoha (2021), the Pseudo-nitzschia pungens species, identified in the Lampung Bay area, was given the LMP3 label. According to Lelong et al. (2012), it can be generally observed that Pseudo-nitzschia is frequently found in high density at a warm temperature, high salinity, and interestingly, in low nutrient concentration. Lampung Bay is situated in the southeast of the island of Sumatra,

with its coastline facing the Sunda Strait, which separates the island from the Indian Ocean.

Potential Implication

The study of *Nitzschia* in the coastal waters of Suak Ribee, West Aceh Regency, Indonesia, can have considerable implications based on the present research, which has shown that morphological surveys can assist in clarifying the taxonomy of *Nitzschia* species, which is key to understanding their ecological roles and evolutionary relationships. This is particularly important for species that are difficult to distinguish based on morphological features. López-Urrutia and Morán (2015), the composition and size structure of phytoplankton can influence primary production rates, thereby impacting the overall energy flow within ecosystems. In addition, variations in phytoplankton community structure can impact the biogeochemical cycling of carbon and other elements, influencing nutrient dynamics within the ecosystem. It is known that certain species of *Nitzschia* produce the neurotoxin domoic acid, which can lead to amnesic shellfish poisoning in humans and mass mortality of wildlife. This includes species within the related genus Pseudo-nitzschia. According to Delegrange et al. (2018), identifying Pseudo-nitzschia and their association with domoic acid levels highlight the significance of implementing monitoring programs for harmful algal blooms (HABs) and toxin production. Regular monitoring can detect the emergence of HABs at an early stage and assess the risk to marine ecosystems and human health.

Conclusion

The Nitzschia genus is known to exhibit a diverse range of species with distinct characteristics. These characteristics include variations in valve width, the presence or absence of a central interspace, fibula and stria density, and other features that distinguish these species. The study of Nitzschia species in the coastal waters of Suak Ribee, West Aceh Regency, Sumatra Island, Indonesia, can provide valuable insights into environmental monitoring and management in the region.

Informed consent

Not available.

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Conflicts of interest

There is no conflict of interests for publishing their study.

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Author contributions

Elya Putri Pane: Writing original draft, Conceptualization, Data curation, Formal analysis, Yenny Risjani: Supervision, Investigation, Methodology, Writing original draft, Yunianta: Superivision, Validation, Methodology, Review, Mehmet Kocabaş: Project administration, Review, Editing, Gilang Drajat Maulana: Formal analysis, Investigation, Luvi S. Handayani: Editing, Review.

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