

# Potential invasive species detection of Demospongiae using environmental DNA in Sabang and Lhokseumawe Ports

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**Abstract.** Sabang and Lhokseumawe Ports are located in the province of Aceh and constitute part of the northern Malacca Strait, sharing direct borders with neighboring countries such as Malaysia, Thailand, and India. Strategic geographical location significantly influences the oceanographic conditions and biodiversity of the area. As marine organisms, sponges play a crucial role in the ecosystem, yet research on their identification in the Sabang and Lhokseumawe regions is limited. The analysis of community structure and identification of species can be conducted using various approaches, including environmental DNA (e-DNA) analysis. This study aimed to assess water quality and identify the presence of potentially invasive Demospongiae using DNA Metabarcoding in Sabang and Lhokseumawe Ports. The measurement results of the water conditions indicate that the waters around Sabang and Lhokseumawe ports generally exhibit values that support sponge life. e-DNA analysis successfully detected the presence of the Demospongiae class, with 260 Operational Taxonomic Units (OTUs) in Sabang waters and 148 OTUs in Lhokseumawe waters. One commonly found genus, *Cliona* sp., has the potential to become invasive in both locations, posing a risk of bioerosion to corals under specific conditions.

## 1 Introduction

Sabang and Lhokseumawe Ports are situated in the province of Aceh and form part of the northern Malacca Strait, directly bordering neighboring countries such as Malaysia, Thailand, and India. Positioning makes the area a crucial international shipping route and a point of entry and exit for foreign vessels [1]. Sabang Island is the outermost island of

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Indonesia, geographically located in the Andaman Sea and adjacent to the Indian Ocean, while the waters of Lhokseumawe are directly connected to the Malacca Strait, a main sea route from the South China Sea to the Andaman Sea. Strategic geographical location plays a significant role in the oceanographic conditions and biodiversity of the area. The high number of foreign vessels passing through the area may pose a threat to the emergence of invasive species facilitated by ballast-water displacement [2].

According to [3], the physical condition of an ecosystem is significantly influenced by the presence and biodiversity of biota in the water body. Sponges are organisms that play a crucial role in the ecosystem. Sponges are marine organisms belonging to the phylum Porifera that contribute to the composition of coastal and marine ecosystems and are commonly found in tropical and subtropical waters [4]. Sponges are immobile, act as filter feeders, and serve as indicators for monitoring marine pollution and interactions within communities [5]. While the majority of Porifera species are harmless, some types can be invasive and potentially have negative impacts on the environment.

Research on sponge identification in the Sabang and Lhokseumawe regions is still limited. Identification and analysis of the community structure of a species can be conducted using various approaches, such as environmental DNA (e-DNA) analysis [3]. DNA extraction from environmental samples, such as soil, sediment, water, or snow, is used to determine the species of an organism [6]. This technology offers high sensitivity and effectiveness, making it crucial as a management strategy for the early detection and monitoring of the presence of biota to develop conservation strategies and protect native species diversity. This study aimed to assess water quality and identify the presence of potentially invasive Demospongiae using DNA Metabarcoding in the Sabang and Lhokseumawe Ports.

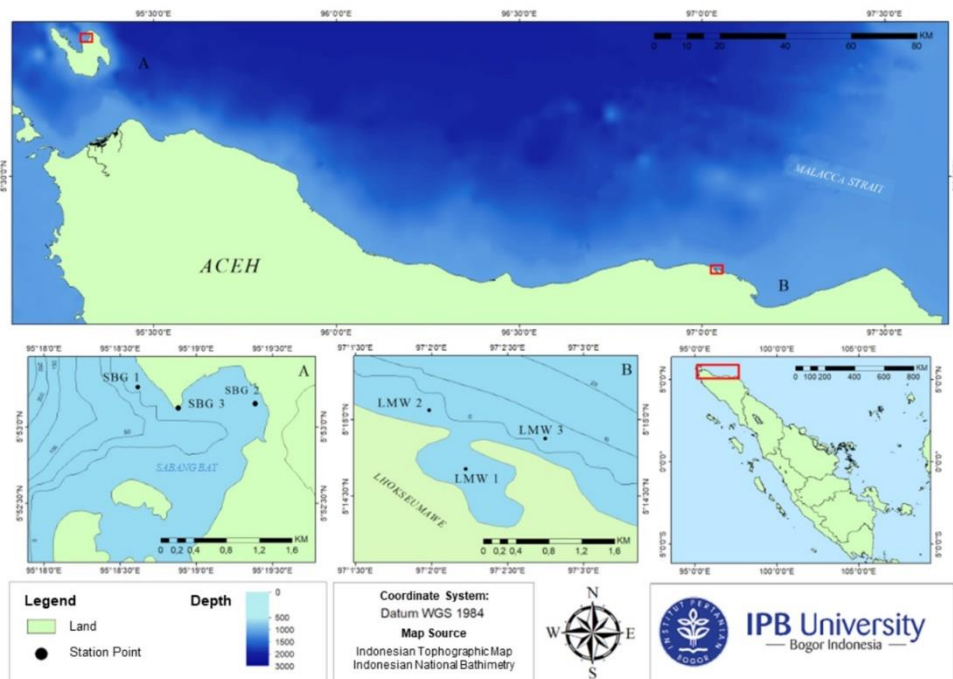
## **2 Material and methods**

### **2.1 Time and location**

Water sampling and in situ data collection were conducted from August 17<sup>th</sup> to 22<sup>nd</sup>, 2021, at three designated observation points at each location, namely Sabang Bay Port (SBG) (Figure 1. A), and Krueng Geukueh Lhokseumawe Port (LMW) (Figure 1. B), and Aceh Province. Sample preservation was performed on-site, immediately after sample collection. Subsequent sample processing and analyses were conducted at the Oceanogen Laboratory in Indonesia.

### **2.2 Tools and materials**

The tools and materials used in water sampling and in situ data collection included a Van Dorn sampler, echosounder, Secchi disk, pH meter, DO meter, hand refractometer, 5-liter jerrycans, and sample bottles. Additionally, for water sample preservation, Nalgene bottles, a vacuum pump, 0.45  $\mu\text{m}$  filter paper, cryogenic tubes, 1 mL DNA/RNA Shield fluid, forceps, scissors, and a pipette were used. Meanwhile, the tools and materials employed in sample processing in the laboratory consist of a centrifuge, vortex mixer, thermocycler, dry block heater, digital scale, measuring cylinders, 0.65 ml microtubes, agar molds, electrophoresis machine, gel doc, UV transilluminator, ZymoBIOMICS DNA extraction kit, 96% ethanol, universal primers, TAQ DNA polymerase, TBE buffer, agarose, and GelRed.



**Fig 1.** The research location map at Sabang Bay Port (A) and Krueng Geukueh Port, Lhokseumawe (B), Aceh Province.

## 2.3 Sample collection

The in situ data collected included water depth and clarity, temperature, salinity, pH (acidity level), and dissolved oxygen (DO). Water depth was assessed through echosounder measurements on the ship, whereas water clarity was measured using a Secchi disk. Temperature values were obtained from measurements displayed on the DO meter, whereas salinity values were measured using a hand refractometer. In situ data collection for pH parameters was conducted using a pH meter, and dissolved oxygen is measured using a DO meter. All instruments must undergo calibration before use to ensure the accuracy of the measurement readings and minimize errors that may occur during the measurement process.

Water samples at each station were collected in the water column at a composite depth of 3-5 meters using a Van Dorn water sampler. Water samples for environmental DNA analysis were a mixture of samples taken from three stations at each location, combined in a 5-liter jerrycan. All samples were then filtered using 0.45  $\mu\text{m}$  filter papers with the assistance of a vacuum pump to expedite the filtration process. The resulting environmental DNA filtrate was carefully folded and transferred into cryogenic tubes containing 1 ml of DNA/RNA shield liquid to prevent degradation of the filtered DNA material.

## 2.4 Lab processing

### 2.4.1 DNA extraction

DNA extraction is a purification method that uses physical and/or chemical methods to separate DNA from cell membranes, proteins, and other cellular components in a sample [7].

DNA extraction involves breaking down cell walls to obtain a pure extract and protecting DNA from degradation [8, 9]. The extraction kit was used in this study.

#### *2.4.2 DNA amplification*

Polymerase chain reaction (PCR) is used to selectively amplify specific DNA segments in vitro. PCR was carried out on a thermocycler with three main steps: (1) denaturation of the dsDNA template at 92–95 °C, (2) primer annealing at 50–70 °C, and (3) extension of dsDNA molecules at approximately 72 °C. Each step was repeated for 30–40 cycles [7]. The universal primers used in this study were designed to detect various types of biotin in the samples.

#### *2.4.3 Electrophoresis*

Electrophoresis is used to separate, analyze, identify, and purify DNA fragments, enabling the visualization of Polymerase Chain Reaction) products [10]. Electrophoresis began with the preparation of a 1.5% agarose gel for DNA visualization. The gel was prepared by mixing 0.75 grams of 1x TAE and 50 ml of TBE buffer [8]. The agarose was then heated in a microwave for approximately 90 s, cooled, and 3 µl of Biotium GelRed was added. Subsequently, 3 µl of the PCR product was loaded into agarose wells, and electrophoresis was conducted at 100 V for 35 min. Electrophoresis results were visualized under ultraviolet light using a UV transilluminator.

#### *2.4.4 DNA sequencing*

DNA sequencing is a method used to determine the nucleotide base sequence of a DNA molecule [11]. DNA can be sequenced using chemical procedures by partially fragmenting DNA molecules labeled with terminals at each base repetition. The DNA sequencing stage in this study was conducted at the Oceanogen Laboratory, and DNA sequencing was performed on an Illumina MiSeq.

### **2.5 Data analysis**

The data obtained from the sequencing results represent the nucleotide base sequence, which was subsequently blasted using the National Center for Biotechnology Information (NCBI) database. The blasting process was performed using the mBRAVE platform. This procedure was performed to identify species based on sequence data and their relationships with species already identified in the NCBI database. Identified organism species were then grouped based on taxonomy and filtered to obtain data on species belonging to the class Demospongiae. Subsequently, the status of the identified species will be checked based on the invasive species database available at the Center for Agriculture and Bioscience (CABI's) Invasive Species Compendium website (<http://www.cabi.org/isc/>) and the Global Invasive Species Database (GISD) (<http://www.iucngisd.org/gisd/>).

## **3 Result and discussion**

### **3.1 In situ water quality**

The diversity of biota in a water body is significantly influenced by environmental conditions and water fertility, which can be determined based on several parameters, such as depth, clarity, temperature, salinity, pH, and dissolved oxygen. The measured values of these

environmental parameters in situ in the Sabang and Lhokseumawe waters are shown in Table 1.

**Table 1.** The in-situ parameter values at each station.

Parameter	Station					
	SBG1	SBG2	SBG3	LMW1	LMW2	LMW3
Depth (m)	30	18	22	27	47	15
Visibility (m)	17	15	12	6.5	9.8	4.1
Temperature (°C)	29.1	29.3	28.38	30	29.9	29.9
Salinity (‰)	32	31	31	31	31	30
pH	8.1	7.9	8	7.8	7.9	7.9
Dissolved Oxygen (mg/L)	6.47	6.33	6.53	6.03	6.51	6.57

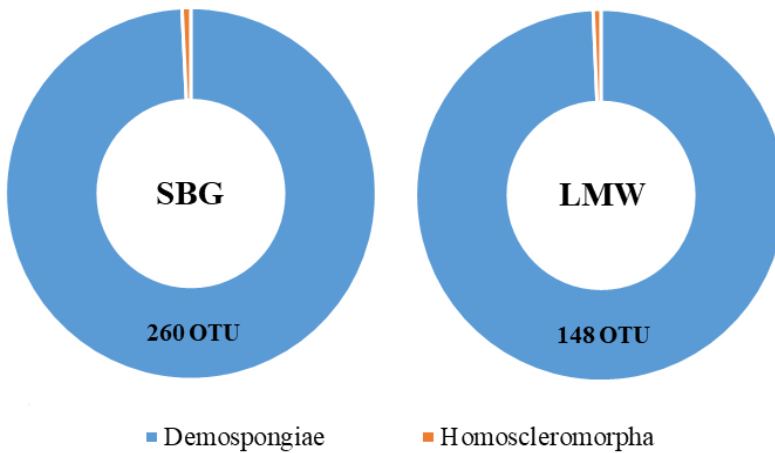
The measurement results indicate that the waters of Sabang Bay have a depth ranging from 22 to 30 m with visibility of 12 to 17 m, whereas the waters of Lhokseumawe have a depth ranging from 15 to 47 m with visibility of 4.1 to 9.8 meters. The depths of both waters were considered optimal, except at station LMW2. Visibility in Sabang waters is considered optimal for sponge life; however, in Lhokseumawe waters, visibility is below the optimal conditions for sponges. Water depth significantly influences other environmental factors; generally, environmental parameter values decrease with increasing depth because the penetration of sunlight into the water column decreases [12].

The temperature values in both waters were considered to be optimal for sponge life. Temperature is a crucial factor that affects sponge reproduction. Sponges tend to be susceptible to stress in conditions with fluctuating temperatures but can adapt better to temperature decreases by reducing respiration. Some sponges harboring zooxanthellae in symbioses are much less affected by increasing temperatures [13]. According to [14], sponges reproduce well at optimal temperatures, whereas an increase in temperature slows down the maturation of sperm and eggs in sponges. The salinity values at both locations are still optimal for the survival of sponge biota, ranging from 31 to 32 ‰ in Sabang waters and 30 to 31 ‰ in Lhokseumawe waters. Salinity in Indonesian waters generally ranges from 28 to 33 ‰, indicating that the salinity conditions at both locations are still within the normal range where marine sponges survive under 35‰ [15]. The concentration of dissolved oxygen in both waters was in accordance with the optimum conditions for sponge life, ranging from 6.03 to 6.57. The acidity or pH ranges from 7.8 to 8.1, which is considered good for marine organisms, but has not yet reached the optimum conditions for sponge life. Stations with optimum pH conditions for sponge growth were SBG1 and SBG3.

### 3.2 Identification of potentially invasive Demospongiae species

OTUs identification from e-DNA samples successfully detected the presence of the Porifera phylum in both locations, with 262 OTUs in Sabang Bay and 149 OTUs in Lhokseumawe. Organisms from the Porifera phylum found at both locations were predominantly of the Demospongiae class, with 260 OTUs in Sabang waters and 148 OTUs in Lhokseumawe waters (Figure 2). Demospongiae, commonly known as sponges, are organisms that can

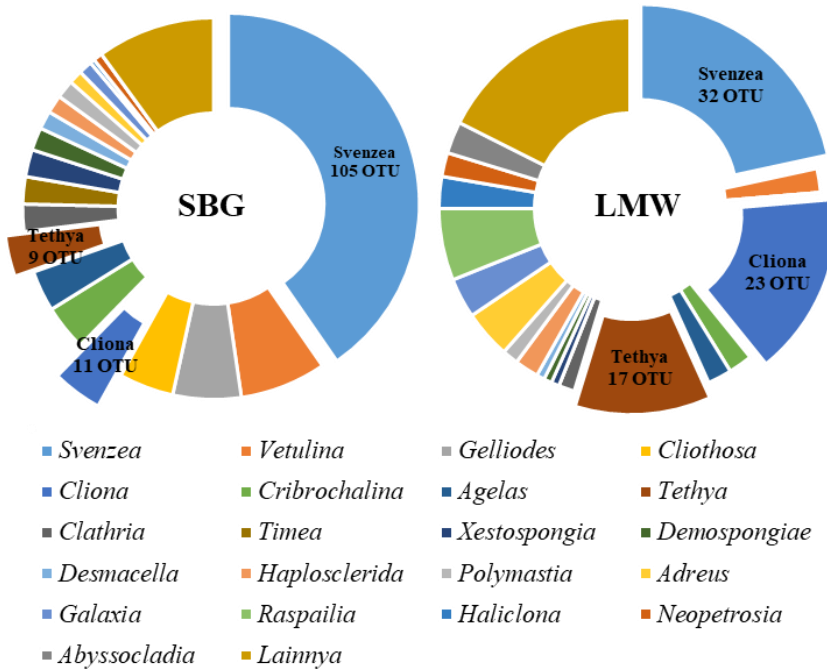
thrive in various habitats with hard structures and are vital components of coastal and marine ecosystems, both in tropical and subtropical waters [16].



**Fig 2.** The composition of Porifera based on the number of OTUs in Sabang (SBG) and Lhokseumawe (LMW) ports.

The most frequently identified sponge species in both locations was *Svenzea* sp., with a total of 105 OTUs in Sabang and 32 OTUs in Lhokseumawe (Figure 3). *Svenzea* sp. is a demosponge genus characterized by a thick crust with chimney-like oscular elevations, colored in a brownish-gray hue [17]. This genus is commonly found at depths of 10–30 m and thrives on open rocky reefs or offshore coral reefs. *Tethya* sp. was also prevalent in both locations, with 9 OTUs in Sabang and 17 OTUs in Lhokseumawe. These sponges have a round to elongated shape and stalked structures, are rigid, and typically appear in gray to white shades. *Tethya* sp. is frequently abundant at depths ranging from 15 to 20 m.

Another genus commonly found in both locations was *Cliona* sp., which was identified in 11 OTUs in Sabang and 23 OTUs in Lhokseumawe. *Cliona* sp., commonly known as boring sponges, are frequently found in tropical waters and typically live on calcareous substrates, creating holes in rocks or mollusk shells. This sponge is adaptable and can thrive in shallow locations owing to its strong attachment ability and effective resistance to currents and waves [18]. Sponges from the *Cliona* genus play a crucial role in maintaining the reef structure by balancing the stored calcium carbonate in the reef [19]. This sponge has the potential to become invasive, as it can cause bioerosion of corals under certain conditions [20]. It is often found in open waters or exposed areas with polyhaline-to-mesohaline water conditions [21].



**Fig 3.** Species composition of Demospongiae in Sabang (SBG) and Lhokseumawe (LMW) ports.

## 4 Conclusion

The conclusions of this research indicate that the water conditions around the Sabang and Lhokseumawe ports generally exhibit values that support sponge life. Through identification using DNA Metabarcoding, the sponge species *Cliona* sp. was detected, indicating its potential as an invasive biota. These findings highlight the importance of careful monitoring and management of invasive species in both locations.

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