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# Revealing Porifera Diversity by Metabarcoding Environmental DNA Data in Coral Reef Ecosystems of Lombok Island Waters

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## ABSTRACT

Lombok waters are located in the Coral Triangle area, a region with high marine biodiversity, including Porifera. Information on Porifera diversity in Lombok waters is still minimal, due to the difficulty of identifying them. Metabarcoding eDNA is an alternative method for revealing the potential diversity of Porifera non-invasively, widely, quickly, and effectively. This study aimed to determine the diversity of Porifera with eDNA data in Lombok Waters. This study used quantitative methods with bioinformatics tools in the data processing process. The eDNA data were obtained from previous research results that have been stored in GenBank PRJNA768103 which includes seawater and sediment sample types in the 12 µm and 0.4 µm membrane pore fractions in the East, West, and North Lombok regions This study successfully identified 19 genera of Porifera dominated by *Hippospongia*, *Haliclona*, and *Terpios* in each location and sample type. Comparison of alpha diversity showed a significant difference in membrane pore fraction ($p = 0{,}0262$). Meanwhile, beta diversity showed a significant difference in the Porifera community between East and North Lombok ($p = 0{,}01$). Significant differences in alpha and beta diversity indicate the influence of environmental factors and habitat characteristics in shaping the community structure of Porifera in Lombok waters. These findings highlight the potential of the eDNA approach as an efficient biomonitoring tool for marine biodiversity and support sustainable management and conservation of Lombok's marine ecosystems.

**Keywords:** *Bioinformatics, Diversity, Environmental DNA, Lombok Waters, Porifera.*

## ABSTRAK

Perairan Lombok berada pada kawasan *Coral Triangle* yang kaya akan keanekaragaman hayati lautnya, salah satunya Porifera. Informasi keanekaragaman Porifera di perairan Lombok masih sangat terbatas, dikarenakan tingkat kesulitan mengidentifikasinya. Metabarcoding eDNA menjadi metode alternatif dalam mengungkapkan potensi keanekaragaman Porifera secara non-invasif, luas, cepat, dan efektif. Penelitian ini bertujuan untuk mengetahui keanekaragaman Porifera berdasarkan data eDNA di Perairan Lombok. Penelitian ini menggunakan metode kuantitatif dengan pendekatan bioinformatika dalam proses pengolahan datanya. Data eDNA diperoleh dari hasil penelitian sebelumnya yang telah tersimpan pada GenBank PRJNA768103 yang mencakup tipe sampel air laut dan sedimen pada fraksi pori membran 12 µm and 0.4 µm di wilayah Lombok Timur, Barat, dan Utara. Penelitian ini berhasil mengidentifikasi 19 genera Porifera yang didominasi oleh *Hippospongia*, *Haliclona,* dan *Terpios* di setiap lokasi maupun tipe sampel. Perbandingan keanekaragaman alfa menunjukkan adanya perbedaan signifikan pada fraksi pori membran ($p = 0{,}0262$). Sementara itu, keanekaragaman beta menunjukkan adanya perbedaan signifikan pada komunitas Porifera antar Lombok Timur dan Utara ($p = 0{,}01$). Perbedaan signifikan pada keanekaragaman alfa dan beta menunjukkan adanya pengaruh faktor lingkungan dan karakteristik habitat dalam membentuk struktur komunitas Porifera di Perairan Lombok. Temuan ini menegaskan bahwa pendekatan *e*DNA berpotensi sebagai alat biomonitoring keanekaragaman hayati laut yang efisien serta mendukung pengelolaan dan konservasi ekosistem perairan Lombok secara berkelanjutan.

**Kata kunci:** Bioinformatika, DNA Lingkungan, Keanekaragaman, Perairan Lombok, Porifera.

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## 1. Introduction

Lombok Island, West Nusa Tenggara Province, is part of the Coral Triangle area (Gelis et al., 2021) which is recognized as the center of the world's marine biodiversity. This area represents about 29% of the world's coral reefs (Ridona, 2015). Coral reefs are one of the coastal ecosystems built by a group of calcium carbonate-producing coral animals symbiotic with zooxanthellae algae (Zurba, 2019). These ecosystems provide habitat, food sources, and protection for many marine life. One of the constituents of the coral reef ecosystem is Porifera.

Porifera are ecologically influential marine invertebrates that can filter dissolved organic matter in water, so they can control nutrient concentrations that affect the distribution of marine biota (Pawlik et al., 2016). Porifera can associate with various organisms, making them the highest biodiversity contributor to coral reef ecosystems (Hadi, 2018). Porifera includes four classes, namely Calcarea, Hexactinellida, Demospongiae, and Homoscleromorpha (Rozanah et al., 2023).

Despite their ecological role, Porifera are often overlooked in biodiversity studies due to the difficulty in identifying them, taxonomically or molecularly (Timmers et al., 2022). Biological diversity can be a major challenge in understanding ecological processes through biomonitoring of a taxonomic group (Gelis et al., 2021). Direct observation requires a long time and great effort and there is a possibility of disturbing the habitat of the target biota. For this reason, indirect observations can be made with an alternative method, namely eDNA metabarcoding.

Environmental DNA is a DNA component derived from a biota shed in the environment (Garlapati et al., 2019), through urine, feces, skin fragments and others. Metabarcoding eDNA is a method of utilizing eDNA from the environment that has been amplified and sequenced to reveal the existence and diversity of biota without isolating it (Taberlet et al., 2012). The advantages of this method are that the sampling process is easy and-, fast, -causes minimal damage to target biota or ecosystems, and can be scaled up for biomonitoring (Anderson, 2001).

However, comprehensive baseline information on Porifera diversity in Lombok Waters is still limited (Sipayung et al., 2025) and the difficulties of morphological and molecular identification further hinder accurate assessments of this group in the region. Therefore, the use of eDNA metabarcoding provides an effective and non-invasive alternative to overcome these limitations, allowing a broader detection of Porifera taxa. Given these gaps, this study aims to characterize the diversity of Porifera in the coral reef ecosystems of Lombok Waters using eDNA metabarcoding. Specifically, this study seeks to (1) determine the diversity of Porifera based on eDNA obtained from seawater and sediment samples across different membrane pore-size fractions, and (2) compare Porifera diversity among sampling locations and sample types within Lombok Waters.

## 2. Materials and methods

This study did not involve the collection of new samples. The data used were secondary eDNA data collected on July 5 - 12, 2018, in the coral reef ecosystem of East, North, and West Lombok waters (Pratomo et al., 2022) (**Figure 1**). Data processing was carried out from October to November 2024 using bioinformatics tools. Secondary data include eDNA data from seawater and sediment samples obtained from GenBank PRJNA768103. This data has gone through the process of extraction, amplification, and sequencing.

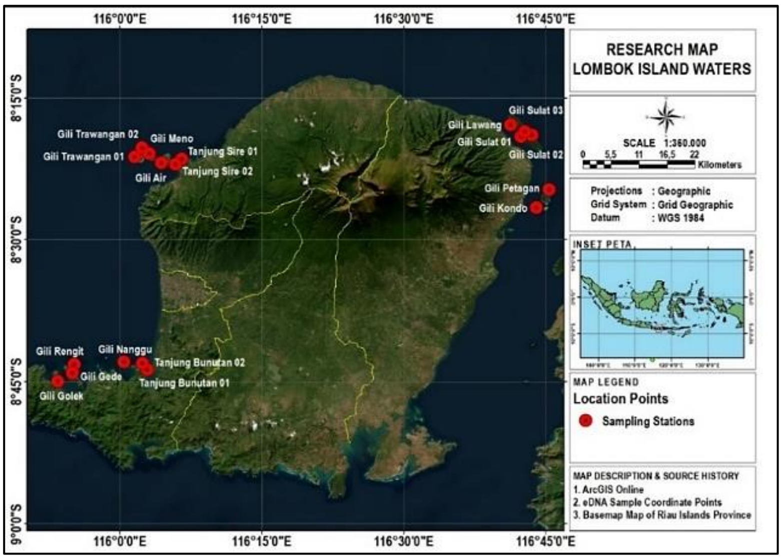

**Figure 1.** Research Location Map

### 2.1. Bioinformatics analysis

The stages of the bioinformatics analysis process in this study are as follows:

1. Data Pre-Processing and Quality Control

The quality of eDNA sequence data was checked using FastQC version 0.12.1. Adapters and short reads < 100 bp with low quality (Q score ≤ 20) were trimmed to minimize interference that could affect the final results (Martin, 2011). Trimming was performed using Cutadapt version 2.8.

2. Taxonomy determination

The clean sequences generated from the Cutadapt process will be denoised with the DADA2 plugin to remove errors in the sequence data that could come from amplification or sequencing errors. The output results were ASVs (*Amplicon Sequence Variants*) as representative sequences. ASV classification was based on the SILVA 132 database as the reference taxon. The output was a taxonomic table containing many taxa. Then, taxa that were not Porifera will be filtered and eliminated. ASV Porifera will be aligned using MAFFT and phylogenetic reconstruction employing FastTree to display the phylogenetic tree.

3. Statistical Analysis

Statistical analysis in the form of alpha and beta diversity analysis was performed on QIIME2. Alpha diversity analysis uses the Shannon index, while beta diversity analysis uses the Bray-Curtis dissimilarity index.

Furthermore, a significance test was carried out with a non-parametric statistical test to determine whether there was a significant difference ($p < 0.05$) in the results of alpha diversity with the H test (Kruskal & Wallis, 1952) and the results of beta diversity with permanova (Anderson, 2001). If there was a significant difference, further tests were carried out with comparison tests between locations, sample types (seawater and sediment), or pore fractions (12 µm and 0.4 µm).

## 3. Results and Discussions

### 3.1. Sequences and Amplicon Sequence Variance (ASV)

Of 41 samples taken at 18 stations, the eDNA data were successfully sequenced using the NGS machine. eDNA samples that are not successfully sequenced can result from eDNA degradation during the process of transporting or extracting samples. The degradation of eDNA can be influenced by environmental factors such as pH and high temperature that can stimulate microbial metabolism, as well as exposure to solar radiation that can damage DNA photochemically (Strickler et al., 2015). According to Kuncoro et al. (2023), sample screening should be done immediately after sample collection to reduce the risk of degradation.

The raw eDNA data produced 3,168,655 demultiplexed sequences with approximately 30,205-240,604 sequences per sample (**Figure 2a**). Then, after denoising using DADA2, the number of sequences became 2,738,264 high-quality sequences with approximately 24,644-199,247 sequences per sample (**Figure 2b**). The total number of ASVs identified from the DADA2 process was 20,554 ASVs. The unique sequences (ASV) resulting from the DADA2 process can be used as individual representatives of a particular species, variant, or phenotype (Pratomo et al., 2022). The ASV classification results showed that there were 5 kingdoms identified in Lombok Waters, namely Chromista (35%), Animalia (10%), Protozoa (6%), Plantae (2%), and Fungi (2%), while 45% of ASVs were not identified to the kingdom level.

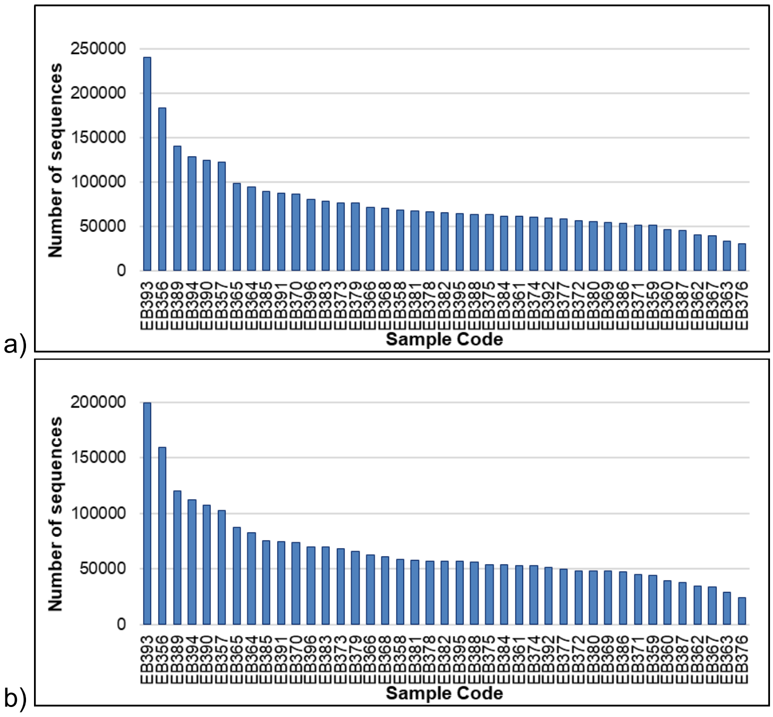

**Figure 2.** Number of Sequences: a) Sequencing Results; b) Denoising Results using DADA2;

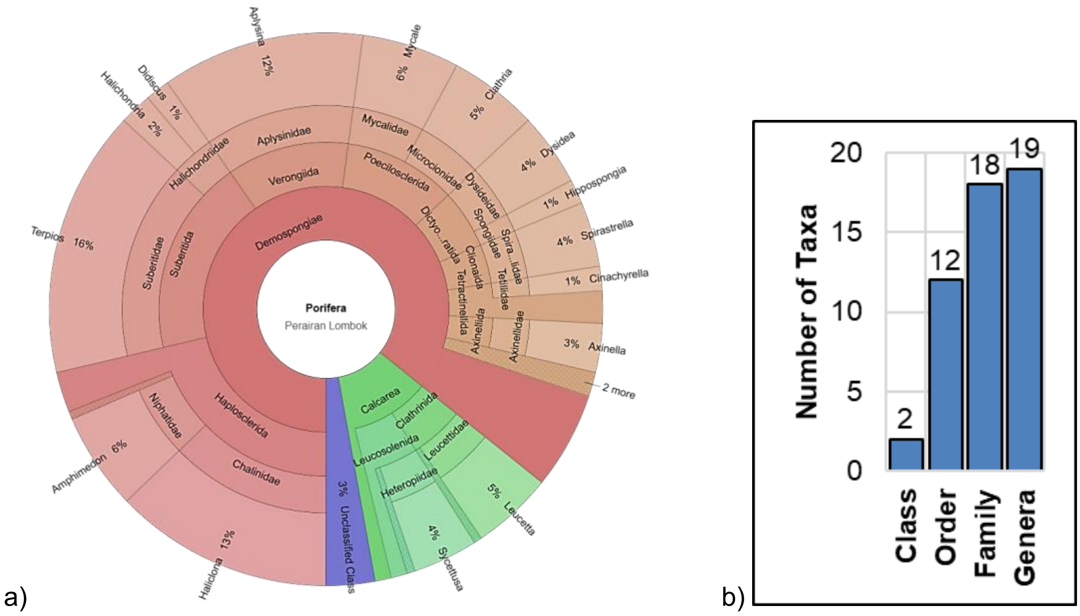

**Figure 3**. Identified Porifera: a) Krona Diagram at Class to Genus Level; b) Total Porifera Taxa Successfully Classified

### 3.2. Identification of Porifera

The number of Porifera ASVs specified in Lombok Waters was 211 ASVs with a total of 47,256 sequences. The dominant Porifera taxa were the Demospongiae class (86%), followed by Calcarea (11%), while (3%) cannot be identified to the class level (**Figure 3a**). According to Soeid et al (2019), the most dominant group of sponges currently and widely distributed in nature with diverse types is the Demospongiae class. About 19 genera of Porifera were successfully classified, including *Amphimedon*, *Anamixilla*, *Aplysilla*, *Aplysina*, *Axinella*, *Cinachyrella*, *Clathria*, *Didiscus*, *Dysidea*, *Halichondria*, *Haliclona*, *Hippospongia*, *Leucetta*, *Mycale*, *Soleneiscus*, *Spirastrella*, *Sycettusa*, *Terpios*, and *Xestospongia* (**Figure 3b**).

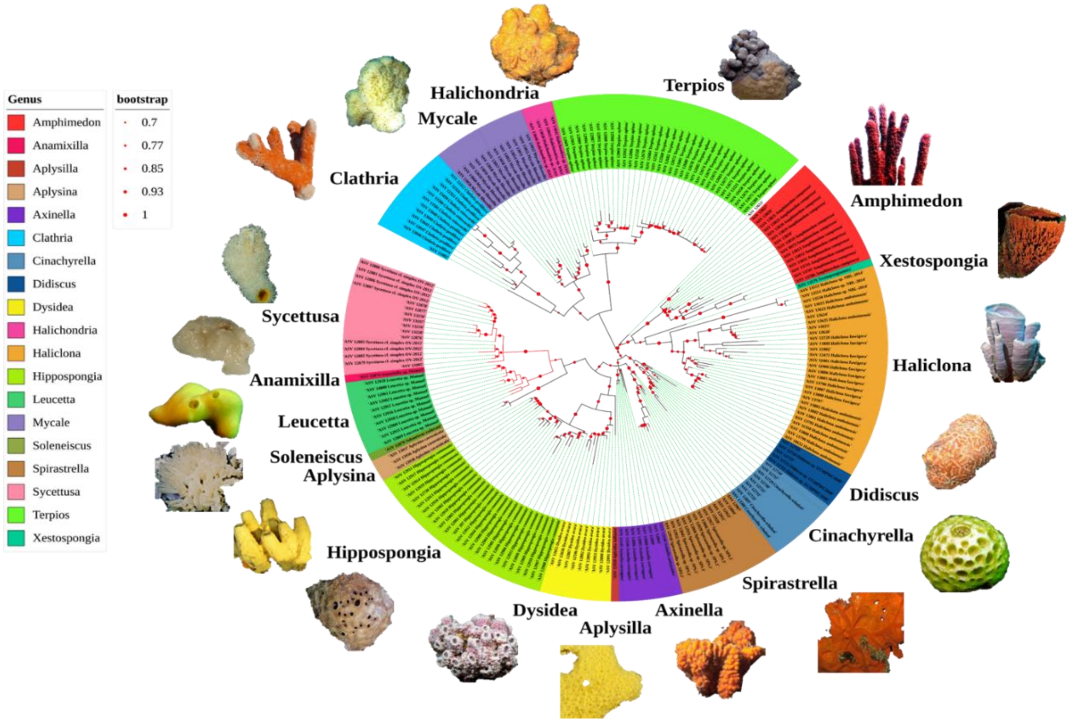

**Figure 4.** Phylogenetic Tree of Porifera showing the Demospongiae (black branch) and Calcarea (red branch) classes.

### 3.3. Evolutionary Relationship of Porifera

Each genus in the phylogenetic tree is grouped with a different color (**Figure 4**). Bootstrap values on the tree branches range from 0.7 to 1. A value >70% indicates the probability that the formation of the tree will not change the composition, making it reliable (Rosidiani et al., 2013). Based on the branching position, Demospongiae appeared earlier at the main branching point compared to Calcarea which appeared as a younger branch. This indicates that Demospongiae is an older relative than Calcarea. This is supported by research by Sperling et al. (2010) which states that Hexatinellida and Demospongiae are the oldest sponge groups and have silica spicules that have evolved since the Precambrian era.

Within the class Demospongiae, genera *Mycale*, *Halichondria*, and *Clathria* share a more recent common ancestor than *Terpios*, but *Mycale* and *Halichondria* show a closer relationship. The genera *Xestospongia* and *Haliclona* are also more closely related and share a more recent common ancestor than *Amphimedon*. The genera *Didiscus* and *Cinachyrella* are also closely related. The genera *Dysidea* and *Hippospongia* also appear closer to each other than *Aplysilla*. Within the class Calcarea, the genera *Anamixilla* and *Sycettusa* are closely related, as are *Leucetta* and *Soleneiscus*. However, these genera share a more recent common ancestor than *Aplysina*.

### 3.4. Distribution of Porifera

Based on the distribution of Porifera at each location (**Figure 5a**), it is known that there are four genera found only in East Lombok, including *Anamixilla*, *Aplysilla*, *Soleneiscus*, and *Xestospongia*. The Venn diagram also shows an overlap or genus found in more than one location. This can be seen in East and North Lombok, which both have the genera *Cinachyrella*, and *Spirastrella*. Likewise, in East and West Lombok, the genus *Sycettusa* was also found. In East, North, and West Lombok, the same 12 genera were found, including *Amphimedon*, *Aplysina*, *Axinella*, *Clathria*, *Didiscus*, *Dysidea*, *Halichondria*, *Haliclona*, *Hippospongia*, *Leucetta*, *Mycale*, and *Terpios*.

Based on the distribution of Porifera in each sample and pore fraction (**Figure 5b**), it is known that in seawater samples with a pore fraction of 0.4 µm, the genera *Anamixilla* and *Aplysilla* are only found in this combination. Likewise in sediment samples (0.4 µm), the genus *Soleneiscus* was only found in this combination. This suggests that technically, using a membrane pore fraction of 0.4 µm in sample filtration will increase the chances of obtaining eDNA biota, including those that may not be detected if a larger pore fraction, such as 12 µm, is used. Overlap was seen in seawater (0.4 µm) and sediment (0.4 µm) samples, where both genus *Xestospongia* were found. Then, in seawater (0.4 µm) and sediment (12 µm) samples, which also both were found the genus *Didiscus*. Likewise, in sediment samples (12 µm) and sediment (0.4 µm), both found the genus *Cinachyrella*. Overlap is also seen in seawater (0.4 µm), sediment (12 µm), and sediment (0.4 µm) samples, where both the genus *Halichondria* can be found. In all sample combinations, namely seawater (12 µm), seawater (0.4 µm), sediment (12 µm), and sediment (0.4 µm), the same 12 genera can be found, including *Amphimedon*, *Aplysina*, *Axinella*, *Clathria*, *Dysidea*, *Haliclona*, *Hippospongia*, *Leucetta*, *Mycale*, *Spirastrella*, *Sycettusa*, and *Terpios*.

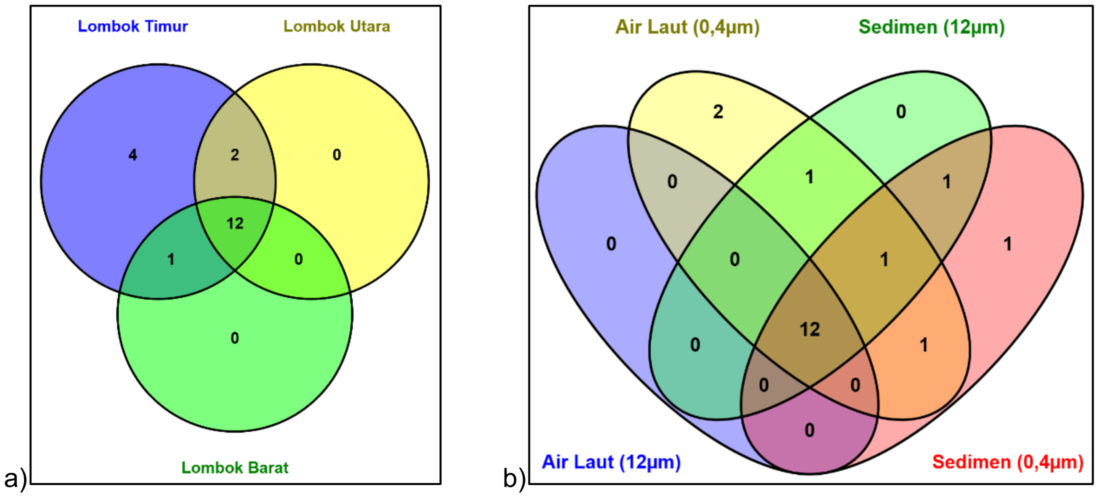

**Figure 5.** Venn Diagram, Distribution of Porifera Found Based on: a) Location; b) Sample Type and Pore Fraction

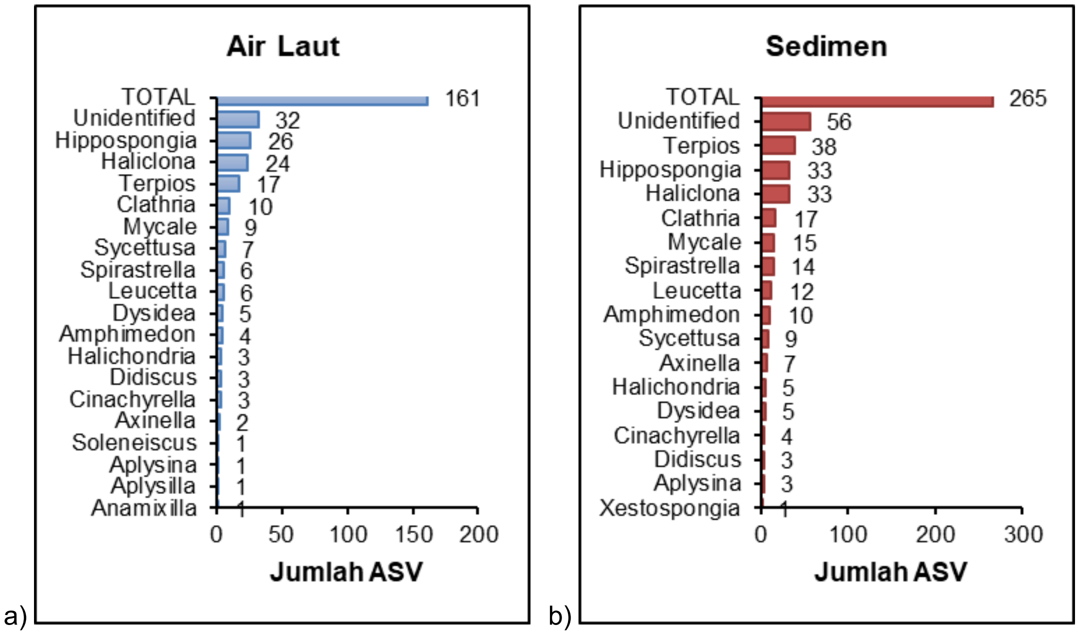

**Figure 6.** Total ASV of Porifera by Sample: a) Seawater; b) Sediment

Regardless of the porous fraction, the number of Porifera genera detected in seawater samples was 18 genera with a total of 161 ASV. Meanwhile, the sediment samples were 16 genera with a total of 265 ASVs (**Figure 6**). The composition of genera found in seawater samples is more diverse than in sediments. However, the total ASV in sediment samples was more than in seawater samples. This means that the distribution of DNA in the environment is wider in seawater, but the concentration of DNA in water is lower. Sediments have higher DNA concentrations than seawater, because the DNA degradation process in sediments is slower due to low temperatures and the absence of exposure to solar radiation (Fonseca et al., 2023).

### 3.5. Relative abundance of Porifera

The rarefaction curves show differences in species richness across the three sites (**Figure 7**). The rarefaction curve depicts a curve line that increases sharply at the beginning and becomes flatter, which means that at the beginning of adding samples, many new species are detected, but with subsequent sample additions, fewer new species are detected (Yurkov et al., 2011). The curve line representing East Lombok shows the highest species richness compared to the other sites. All of the curves have reached asymptotes or flattened, indicating that the amount of sampling done is sufficient to represent Porifera species richness at each location. Increasing the sample size will probably not result in significant new species discoveries.

Based on the barplot in **Figure 8a**, the relative abundance in the East Lombok region is dominated by the genus *Hippospongia* with a proportion of around 17.1%, followed by *Haliclona* with around 9.9%. In the North Lombok region, the genus *Hippospongia* remains dominant with a proportion of around 19.4%, followed by *Terpios* at around 16.3%. The West Lombok region shows a different composition, where this region is dominated by the *Haliclona* genus with a proportion of around 32.8%, while the proportion of *Hippospongia* drops to 14.5%.

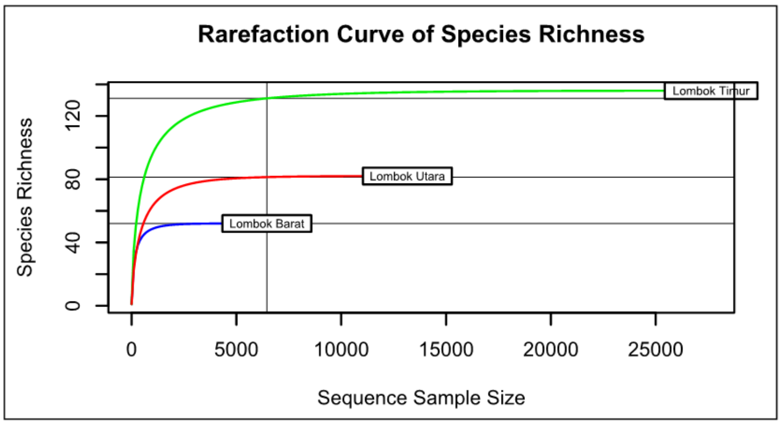

**Figure 7.** Rarefaction Curve at Each Location

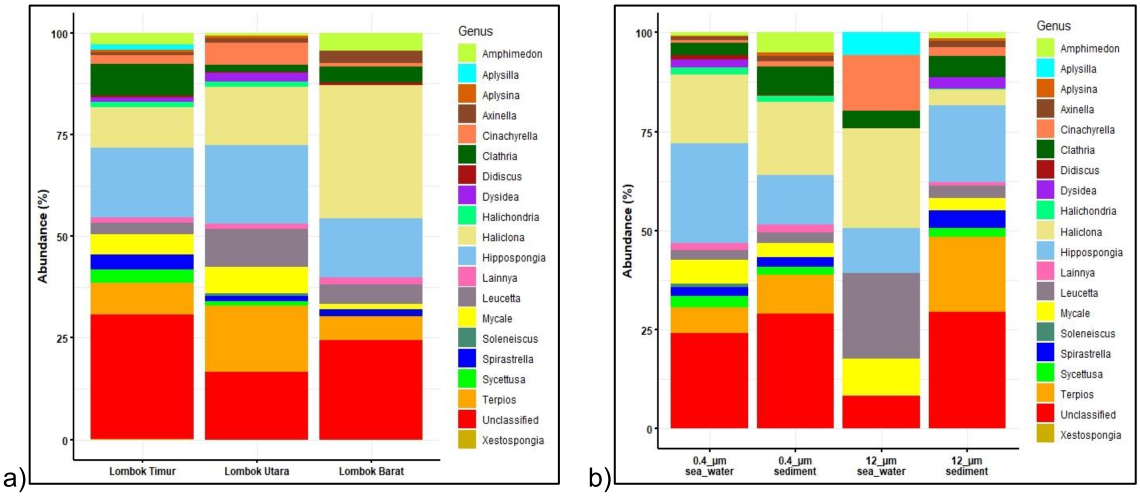

**Figure 8.** Relative Abundance of Porifera Based on: a) Location; b) Sample Type and Pore Fraction

Based on **Figure 8b**, the relative abundance in seawater samples (12 µm) is dominated by the genus *Haliclona* (25.3%), followed by *Leucetta* (21.6%). While in seawater samples (0.4 µm), there is an increase in the genus *Hippospongia* with a proportion of about 25.3%, while the proportion of *Haliclona* dropped to 17.4%. In sediment samples (12 µm) dominated by the genus *Hippospongia* (19.3%), followed by *Terpios*, about 19.1%. Whereas in sediment samples (0.4 µm) show a different composition, where it is dominated by the genus *Haliclona* (18.6%), followed by *Hippospongia* around 12.4%.

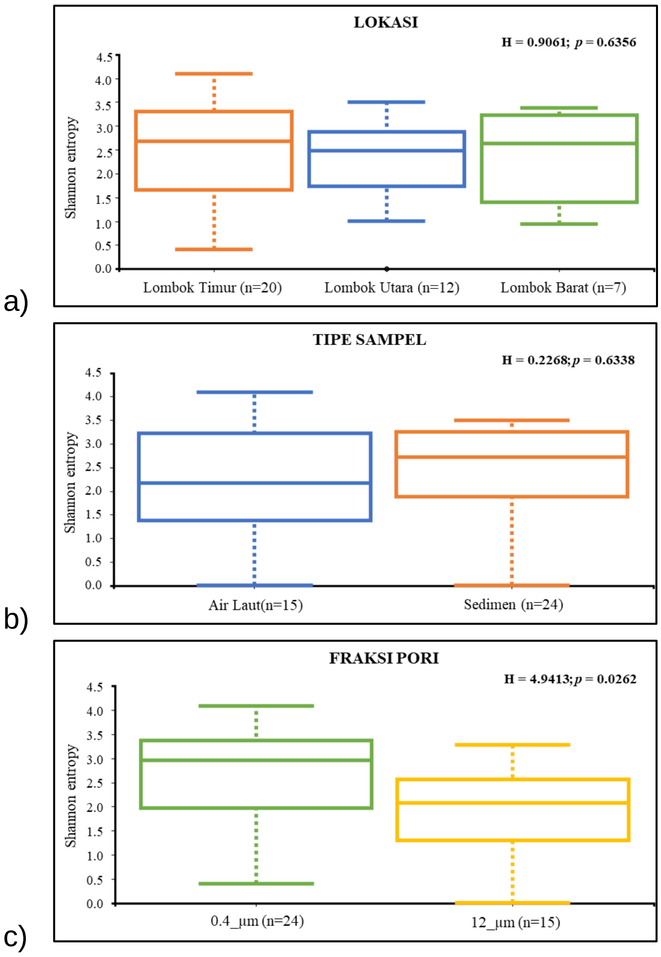

**Figure 9.** Barplot of Kruskal-Wallis Test Results Based on: (a) Location; (b) Sample Type; (c) Membrane Pore Fraction

### 3.6. Alpha and Beta Diversity

Shannon index values for alpha diversity in each sample ranged from 0 to 4.09, with a median of 2.49. Kruskal-Wallis test results showed no statistically significant differences in each location (*p* = 0.6356) and sample type (*p* = 0.6338). The median Shannon index values were relatively similar across regions, with East Lombok at 2,7, North Lombok at 2,5, and West Lombok at 2,6. Similarly, the median values between seawater (2,3) and sediment samples (2,7) showed minimal variation (**Figure 9a and 9b**). This means that geographical location and sample type do not affect the diversity of Porifera in Lombok Waters. However, the pore fraction showed a significant difference (*p* = 0.0262). Samples filtered with 0,4 µm membranes showed higher median Shannon index values (median = 3) compared to those filtered with 12 µm membranes (median = 2,1) (**Figure 9c**). This means that the size of the pore fraction will affect the diversity of Porifera.

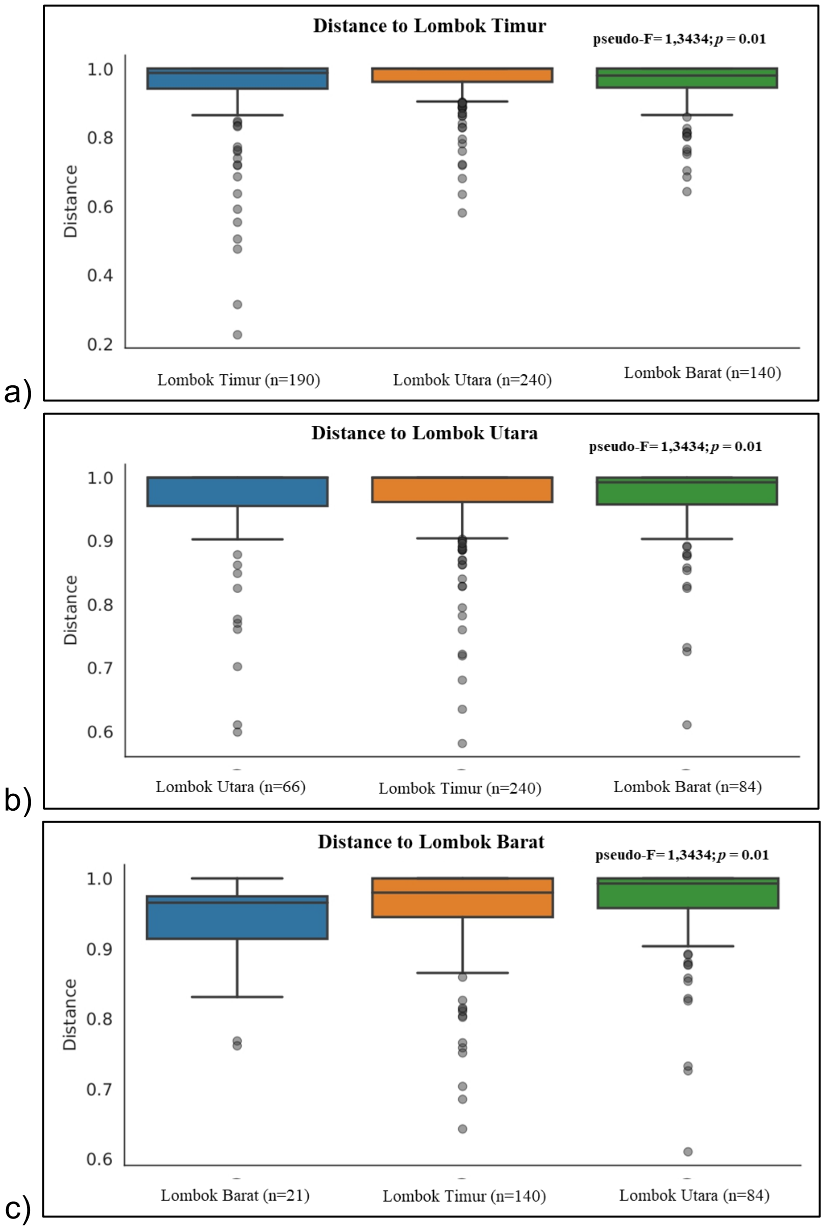

**Figure 8.** Barplot of Permanova Permutation 999 Based on Location: a) East Lombok; b) North Lombok; c) West Lombok

**Table 1.** Results of Further Test Comparison Between Locations

| Group 1 | Group 2 | Sample size | pseudo-F | *p*-value |
|---|---|---|---|---|
| Lombok Timur | Lombok Utara | 32 | 1,476493 | 0,029 |
| Lombok Timur | Lombok Barat | 27 | 1,244922 | 0,111 |
| Lombok Utara | Lombok Barat | 19 | 1,257051 | 0,09 |

The Bray-Curtis dissimilarity index values for beta diversity in each sample ranged from 0.23 to 1.00 with a median of 0.99. This value indicates high heterogeneity in the Porifera community in Lombok waters. Permanova test results showed a significant difference in the Porifera community between locations (*p* = 0.01) (**Figure 10**), namely between East Lombok and North Lombok (**Table 1**). However, the Permanova test results did not show any significant differences in the Porifera community between sample types and membrane pore fractions (**Figure 11**). Groups of points that are close together on the plot indicate similarities in community structure. Whereas, the distance between some other groups of points indicates differences in community structure (**Figure 12**).

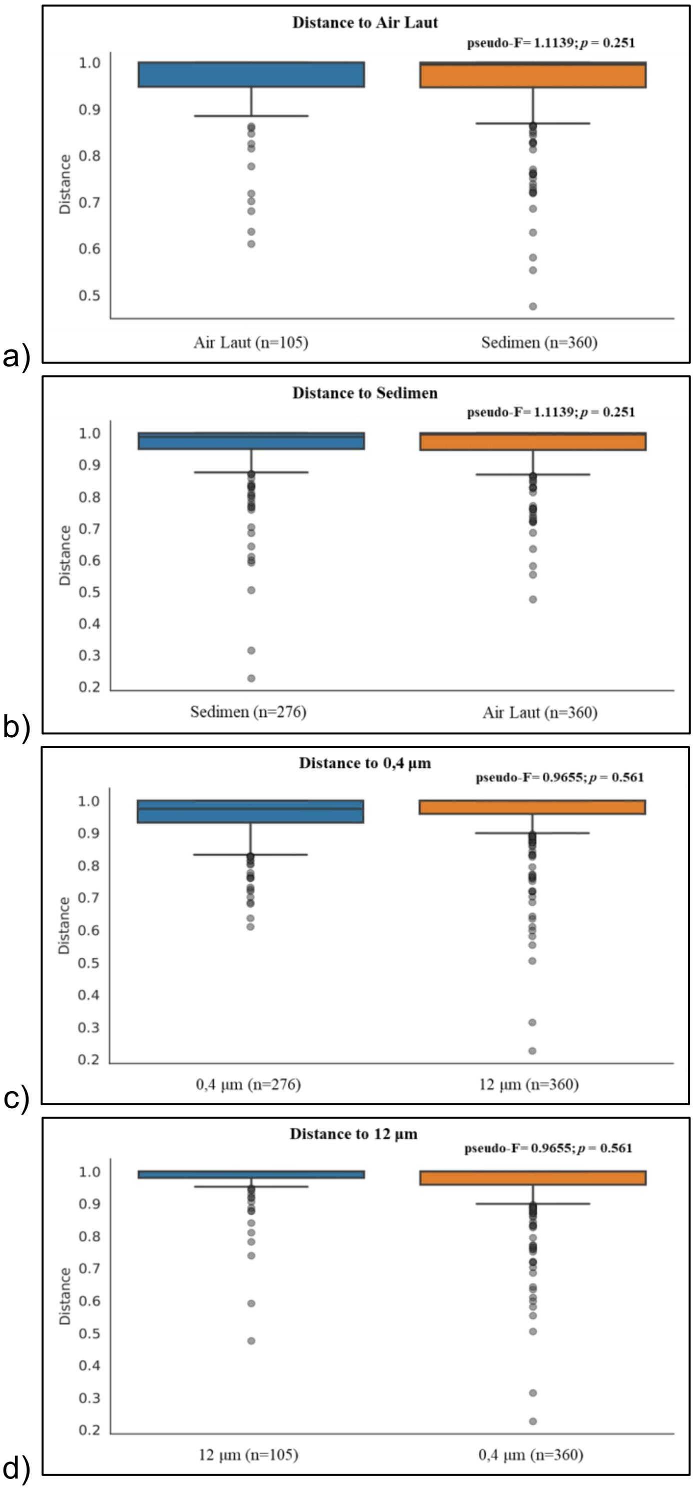

**Figure 9.** Barplot of Permanova Test Results by Sample Type and Pore Fraction: a) Sea water; b) Sediment; c) 0.4 µm; d) 12 µm

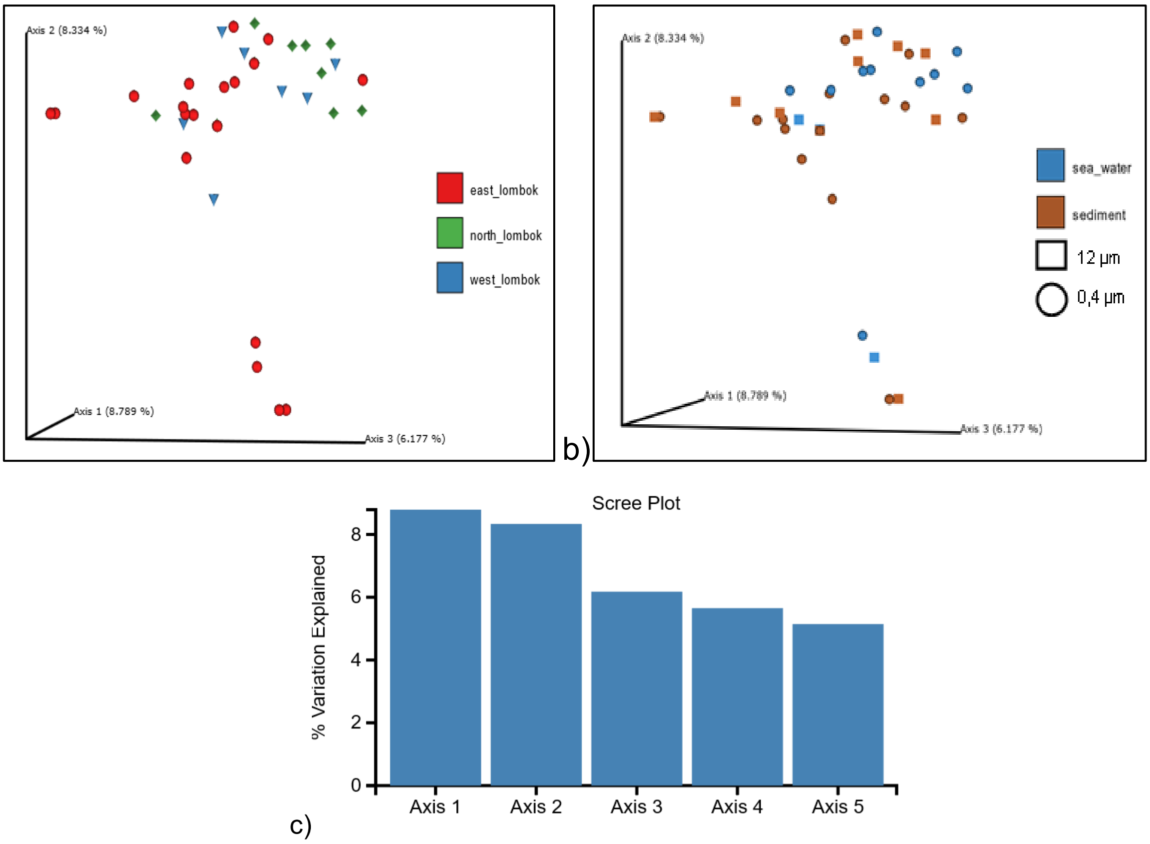

**Figure 10.** Bray-Curtis dissimilarity on PCoA plot diagram: a) Location; b) Sample Type and Pore Fraction; c) Scree Plot Percentage Variation Chart

## 4. Conclusion

The results of the eDNA survey demonstrate a strong potential for non-invasively uncovering Porifera diversity in Lombok waters. While alpha diversity appeared relatively consistent across locations and sample types, beta diversity revealed clear differences in community composition between regions, particularly between East and North Lombok. These findings confirm the effectiveness of eDNA as a tool for biodiversity assessment and highlight the ecological variability of Porifera within the Coral Triangle.

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