Diversity and distribution of Symbiodiniaceae detected on coral reefs of Lombok, Indonesia using environmental DNA (eDNA)

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Abstract

Background Dinoflagellates in the familySymbiodiniaceae are important to coral reef ecosystems, including contributing to coral health and growth. Few studies have been conducted to investigate the diversity and distribution of Symbiodiniaceae in the region of Indonesia. Understanding the distribution of different kinds of Symbiodiniaceae may improve forecasting the future responses of various coral reef systems to climate change. The objective of this study was to develop a rapid proxy for estimating the diversity of Symbiodiniaceae in the reef waters around Lombok Island (east, north, and west) using environmental DNA (eDNA), and to compare the results across sampling sites, sample type (seawater/sediment) and filter pore-size (fraction).

Methods Seawater and sediment samples were collected from 18 sampling locations and filtered to obtain fractions of 0.4–12 µm and >12 µm. After extraction, molecular barcoding PCR amplified the primary V9-SSU 18S rRNA gene, followed by sequencing (Illumina-MiSeq). BLAST, Naïve-fit-Bayes, and maximum likelihood routines were used for classification and phylogenetic reconstruction.

Results Phylogenetic analysis resolved the amplicon sequence variants (ASVs) obtained into 16 subclades comprising 6 Symbiodiniaceae genera (or genera-equivalent clades): *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, Foraminifera Clade G, and *Halluxium*. Comparative analysis showed that three distinct lineages within *Cladocopium*, *Durusdinium*, Foraminifera

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Clade G were most common. Most of the sequences recovered appeared to be were distinctive of different sampling locations, supporting the possibility that eDNA may resolve regional and local differences in the prevalence of certain symbiodiniacean genera and species.

Conclusions This study indicates that eDNA surveys may offer a rapid proxy for evaluating Symbiodiniaceae diversity across different coral reefs around the world, and can therefore be a useful approace to enhance understanding of the diversity and relative ecological dominance of certain Symbiodiniaceae. Moreover, the analysis of Symbiodiniaceae eDNA support the potential of monitoring the local and regional stability of coral-algal mutualisms.

Subjects Marine Biology, Biodiversity, Ecology, Molecular Biology, Genetics

Keywords Coral Triangle, endosymbiotic dinoflagellate, plankton, benthic periphyton, next generation biomonitoring

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INTRODUCTION

Endosymbiotic dinoflagellates in the family Symbiodiniaceae are extremely prevalent in coral reef ecosystems. Also known as zooxanthellae, these endosymbionts play vital roles such as providing energy, absorbing residual metabolites, and promoting growth of their coral hosts (Davy, Allemand & Weis, 2012; Purnomo, 2014). These symbionts also contribute to the adaptability and resilience of coral communities to environmental change, in particular the impacts of ocean warming (Berkelmans & Van Oppen, 2006; Baskett, Gaines & Nisbet, 2009; Suggett, Warner & Leggat, 2017; Claar et al., 2020; Howells et al., 2021). Stress tolerant Symbiodiniaceae can improve the survival of coral colonies exposed to thermal (; Bourne, Morrow & Webster, 2016). Therefore, understanding the potential diversity of Symbiodiniaceae is necessary as part of the broader effort to forecast the future of coral reef ecosystems in different regions.

Symbiodiniaceae engage in mutualistic relationships with various invertebrates besides corals, including jellyfishes, molluscs, sponges, flatworms, and foraminifera (LaJeunesse et al., 2010, 2018; LaJeunesse, Pochon, Putnam & Gates, 2014). Meanwhile, a number of Symbiodiniaceae live as aquatic plankton and benthic periphyton, and some are associated with macro-algae and seagrasses (Venera-Ponton et al., 2010; Takabayashi et al., 2012; Fujise et al., 2021).

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Currently, there are 11 recognized named genera in the Symbiodiniaceae family: *Symbiodinium* (clade A), *Philozoon* (temperate Clade A), *Breviolum* (clade B), *Cladocopium* (Clade C), *Durusdinium* (Clade D), *Miliolidium* (Foraminifera clade D), *Effrenium* (clade E), *Freudhentalidium* (clade Fr3), *Fugacium* (clade Fr5), *Gerakladium* (clade G), and *Halluxium* (clade H) (LaJeunesse et al., 2018, 2021; Nitschke et al., 2020; Pochon & LaJeunesse, 2021). However, LaJeunesse et al. (2018) proposed 15 distinct lineages, which remain clades those are Foraminifera Clade G, Clade Fr2, Clade Fr4, and Clade I represent undescribed genera.

Although Indonesia is part of the Coral Triangle (Veron et al., 2009; Gelis et al., 2021) and coral reef ecosystems are a valuable economic resource for most coastal communities across the Indonesian Archipelago, data on the diversity of Indonesian Symbiodiniaceae are still limited (Loh et al. 2006, Bo et al. 2011, Purnomo 2014, DeBoer et al. 2012). On the other hand, many past studies about Symbiodiniaceae taxa from areas around Indonesia such as the South China Sea, Thailand, Singapore, Palau, the Philippines, and Timor-Leste. Most of These studies focused on Symbiodiniaceae populations within host organisms. Some Symbiodiniacean genera reported of those were *Symbiodinium, Breviolum, Cladocopium, Durusdinium, Gerakladium*, and *Fugacium* (Fabricius et al., 2004; Loh, Cowlishaw & Wilson, 2006; LaJeunesse et al., 2010; Taguba, Sotto & Geraldino, 2016; Tong et al., 2018; Brian, Davy & Wilkinson, 2019).

It cannot directly identify Symbiodiniaceae using conventional microscopy. The need for collection and isolation from multiple locations increases the difficulty of assessing this

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taxonomic group. Advances in the use of environmental DNA (eDNA) and multi-taxon sequencing techniques (metabarcoding) have made it possible to study Symbiodiniaceae communities through the collection of environmental samples such as water and sediment (Arif et al., 2014; Shinzato et al., 2018; Fujise et al., 2021). The advantages of the eDNA-based approach include its ease of use, non-invasive nature, broad spatial scale, and cost-effectiveness (Deiner et al., 2017).

The objective of this study was to determine whether or not eDNA surveys could describe the diversity of Symbiodiniaceae from water and sediment in the coral reef ecosystems around Lombok Island in Indonesia and to make comparisons across locations, sampling methods (seawater/sediment), and filter pore-size (fraction). The diversity of Symbiodiniaceae determined from the environment can reflect its communities present in coral on the reef. Expectedly, these data will serve as beneficial information for future coral reef research and management.

METHODS

Study Site

The study was conducted in coral reef habitats around Lombok Island, West Nusa Tenggara Province, Indonesia. This Island is the constituent of the marine ecoregion of Nusa Tenggara (Lesser Sunda), which has a coral reef area of about 272,123 ha (Giyanto et al., 2017). The

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Western part of Lombok Island is directly adjoint to Lombok Strait and in the Southern part is the Indian Ocean. The study areas are located 5-100 m from shore, and each of its depths are in the range of about 1 to 10 m based on the lowest low water level with a mean tidal range of about 186 cm. The study was carried out from 5th to 12th July 2018 (see Table 1.).

eDNA sample collection

eDNA seawater and sediment samples were collected by scuba diving from six reef stations within each coastal area or subregion (West Lombok, East Lombok, and North Lombok) during the survey. To do so, a day, six samples were collected from three stations (Fig. 1, Table 1, and Table 2). The distance between sampling stations was at least 1500 m to avoid overlap in sampling. At each reef station, a 4 L seawater sample from the water column (~2 m above the reef substrate) and the sediment (water + sediment in 1:1 ratio) was taken using a sample sterilized bottle. Before sampling every different site, the bottle was rinsed with a 30% commercial bleach solution followed by distilled water rinses. After eDNA sampling, samples were stored in a cool box and brought as soon as possible to basecamp at Lombok Island (less than 12 hours). Each sample was filtered twice using a peristaltic pump (Fisher Scientific) through 47 mm diameter polycarbonate (PCTE) membrane filters (Sterlitech) with two different pore sizes: firstly 12 µm and then 0.4 µm. According to Turner et al. (2014), a combination of 0.2 µm filtration or larger pore size and water volume enables optimal eDNA capture and could

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maximize detection probability. Additionally, a larger pore size filter is required to avoid clogged filters, frequently smaller ones. Particularly sediment samples, it was shaken first and then had just been filtered 1-2 minutes after shaking. Each filter was cut into two, and each half was places in a 1.5 mL vial pre-filled with DNA Shield as a preservative. At the end of the eDNA survey activities, via commercial courier service, all of the samples were transported to the Marine Biodiversity and Biosystematics laboratory at IPB University and then stored at −20 °C until DNA was extracted.

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DNA extraction, amplification, and sequencing

The filtered eDNA samples were extracted and amplified at the Marine Biodiversity and Systematic Laboratory of IPB University and sequenced at the University of Rhode Island (URI) Genomics and Sequencing Center. Briefly, DNA was extracted from filters using ZymoBiomics Miniprep Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. V9 hypervariable regions of the eukaryotic Small Sub Unit (SSU) 18S ribosomal RNA (rRNA) genes were amplified using a PCR platform and prepared for 2 × 250 bp paired-end Illumina MiSeq sequencing (Illumina, San Diego, CA, United States). The V9 primer set 1389F: 5'-TTG TAC ACA CCG CCC-3' and 1510R: 5'-CCT TCY GCA GGT TCA CCT AC-3' (Amaral-Zettler et al., 2009; Stoeck et al., 2010), were used for amplification, with the addition of Illumina adapters, linker sequences, index, and pad (Kozich et al., 2013). The PCR profile used

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was: 3 min at 94 °C, followed by 35 cycles of 94 °C for 45 s, 48 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Each 49 µL PCR reaction comprised 25 µL MyTM HS red Mix (Bioline Ltd., London, UK), 1 µL (10 µM) forward primer, 1 µL (10 µM) reverse primer, 1 µL DNA template, and final volume was adjusted to 49 µL using ddH2O. Final master mix concentration in 1x reaction: 0.8x and final primer concentration in 1x reaction: 0.2 µM. The PCR product was checked using electrophoresis of 5 µL aliquots on 1% agarose gel in 0.5X TBE buffer. Library preparation and sequencing were performed at URI. Briefly, a second PCR was performed to add the dual indices and Illumina sequencing adapters from the TruSeq PCR-Free LT kit to the target amplicons, using Kapa HotStart HiFi 2x ReadyMix DNA polymerase (Kapa Biosystems Ltd., London, UK). The PCR profile used was: initial denaturation at 95 °C for 3 min., 9 cycles of 95 °C for 30 s, 55 °C for 30 s, and final extension at 72 °C for 5 min. The presence and length (bp) of the PCR product or amplicon was tested by electrophoresis. Successful amplicons were then purified using paramagnetic KAPA pure beads (beads-to-sample volumetric ratio in 1.6:1). A Qubit fluorometer with Qubit dsDNA HS Assay reagent (Invitrogen, California, US) was used to quantify all libraries. The prepared samples were combined in equal concentrations and then pooled with a 20% denatured and diluted PhiX Illumina control library. The final pooled library was sequenced on an Illumina MiSeq with the MiSeq v2 500-cycle kit (Illumina, San Diego, CA, United States).

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Data Processing and Bioinformatics Analysis

The forward and reverse raw sequence data obtained were converted to demultiplexed fastq files (see additional information on data availability). The sequence read quality was checked using FastQC v.0.11.8 (https://www.bioinformatics.babraham.ac.uk) at each step in the analysis process. The Cutadapt v.1.18 (Martin, 2011) application was used to trim the reverse and forward primer sequences, remove short reads with lengths < 100 bp and low-quality reads with a Phred Q score of < 20. The Qiime2.2019.10 pipeline (Caporaso et al., 2010; Bolyen et al., 2019) was used for further data processing. In brief, DADA2 v.2018.11.0 (Callahan et al., 2016) (via q2□dada2) was used for denoising, joining denoised paired-end reads, filtering out chimeric sequences and singletons, then dereplicating sequences to produce amplicon sequence variants (ASVs). Due to the high-quality of the sequences obtained after running Cutadapt, trimming and truncating were not performed during the DADA2 processing.

ASVs identification

Identifying Symbiodiniaceae species from the eDNA sequences, the first step was to classify all ASVs (Supplemental File S1) using the q2□feature□classifier (Bokulich et al., 2018) classify-sklearn Fit-Naïve Bayes taxonomy classifier against the 18S NR SILVA (release 123 Qiime compatible) 97% and 99% OTUs reference sequence (https://www.arb-silva.de/download/archive/qiime/). Putative Symbiodiniaceae OTUs were then filtered from the

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Eukaryote taxonomy table obtained (Supplemental File S2). The putative Symbiodiniaceae ASVs (see Table 3. in the second column) were then assessed using the NCBI BLAST routine, selecting the best hit at >95% identity in the nr/nt database of the NCBI (https://www.ncbi.nlm.nih.gov/). The BLAST results (Supplemental File S3) were evaluated, and reference sequences (accessions) were selected to further analyses. Additional SSU 18S Symbiodiniaceae reference sequences (accessions) were obtained from the NCBI database representing several families in the Order Suessiales, Symbiodiniaceae as well as from the V9-SSU 18S sequence reference database of the TARA Ocean Expedition (Decelle et al., 2018) and Loh et al. (2006). The final reference sequence database compiled (Supplemental File S4) contained 82 sequences. These reference sequences and the putative Symbiodiniaceae ASVs from the samples were then aligned in MAFFT v.7 (Katoh & Standley, 2013)) (via q2-alignment) followed by a masking process (Rajan, 2012). A phylogenetic tree representing the evolutionary relationships of the Symbiodiniaceae was constructed using the maximum likelihood (ML) approach in the IQ-TREE v.1.6.12 (Nguyen et al., 2015) (via q2-phylogeny) with 1000 bootstraps. For the IQ-TREE algorithm parameters setup used, please see the relevant analysis code in additional information on data availability. These parameters setup will calculate phylogenetic branch support scores based on the Shimodaira and Hasegawa approximate likelihood ratio test (SH-alrt), with local bootstraps (lbt), approach to Bayesian (abayes), and ultrafast bootstraps (ufboot). Detailed explanations for these scores are provided in

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the IQ-TREE documentation (Minh et al., 2021). The best-fit substitution model was TIM3 + F + R3, chosen according to the Bayesian Information Criterion (BIC) by ModelFinder applied in IQ-TREE (Kalyaanamoorthy et al., 2017). The Symbiodiniaceae taxonomic nomenclature refers to LaJeunesse et al. (2018). The term subclade was used instead of species since the 18S short eDNA sequence does not resolve to species-level for Symbiodiniaceae.

Statistical Analysis

Relative abundance data for the putative Symbiodiniaceae taxa (Supplemental File S5) were yielded from DADA2 resulting and used as data input for the Venn diagram and statistical analyses. Venn diagram analysis was performed using the online application in http://bioinformatics.psb.ugent.be/webtools/Venn/. These analyses compared the Symbiodiniaceae present by location (coastal area), medium (seawater and sediment samples), and fraction (filter pore size). Venn diagram analysis was used to identify widely distributed subclades (common) and specific subclades to each location and/or station (unique) and also to evaluate the success of the various sampling method combinations in detecting Symbiodiniaceae. Statistical analyses were used to compare Symbiodiniaceae abundance, diversity, and features observed across different sites, sample types, and fractions. Alpha diversity (features observed and Shannon's Entropy) and beta diversity (Bray-Curtis dissimilarity) were estimated using q2□diversity after samples were rarefied (subsampled without replacement) to 28 sequences per

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sample. Kruskal-Wallis test (Kruskal & Wallis, 1952) was used to compare groups of abundance, alpha diversity values via q2-diversity alpha-group-significance and Permanova test (Anderson, 2001) to compare groups of beta diversity values via q2-diversity beta-group-significance.

RESULTS

Obtaining sequences, ASVs, and Eukaryote classification

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Out of 72 samples from 18 stations, DNA was successfully extracted from 41 samples from 16 stations, yielding a total of 3,168,655 raw sequences with 30,205 to 240,604 sequences per sample (Table 2 and Fig. S1). The process of DADA2 yielded a total of 20,486 ASVs (Supplementary File S1). The mean length of the sequences obtained was 127.81 ± 22.03 bp. The ASVs classification demonstrates the potential diversity of Eukaryotes in the reef waters of Lombok Island. Based on the total ASVs and taxon level 4 out of 15 taxon levels according to the SILVA database, it appears that the proportion of unclassified Eukaryotes (43.35%) dominates the proportion of Eukaryote taxa. It is followed by Metazoa (9.47%), Ochrophyta (7.83%), Dinoflagellates (4.5%), Discicristata (4.4%), and in detail results can be seen in Fig. 2.

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Symbiodiniaceae detection and classification

Table 3 summarizes the results of Symbiodiniaceae classification performed using a Eukaryote classifier (Supplemental File S2) followed by BLAST (Supplemental File S3) and phylogenetic analysis. The probabilistic classifier detected and classified Symbiodiniaceae taxa to the family level. It results in 22 ASVs (named OTU.sym1 to OTU.sym22) that are putative as Symbiodiniaceae, with confidence levels ranging from 0.743–0.999 (Table 3). The BLAST results indicated that some of these ASVs were neither included in the Symbiodiniaceae nor classified. A partial phylogenetic reconstruction of the families in the Order Suessiales using reference sequences obtained from the databases searched (Supplemental File S4) and the putative Symbiodiniaceae ASV sequences from this study (Fig. 3A), only 16 out of the 22 ASVs resolved within the monophyletic group of the Symbiodiniaceae family clade based on the score of 100/100/1/99 for SH-alrt/lbt/abayes/ufboot respectively. Three of the six remaining ASVs resolved within clades representing genera in the Family Suessiaceae, two ASVs in the *Yihiella* clade (OTU.sym11 and OTU.sym13) and one in the *Ansanella* clade (OTU.sym20). The remaining three resolved within the Suessiaceae family but were not classified to genus-level (OTU.sym3, OTU.sym9, and OTU.sym14).

The Symbiodiniaceae family branch (Fig. 3B) comprised six clades, each representing one genus with strong to moderate support (see the scores in Table 3 and Fig. 3). This phylogenetic topology is concordant with the Symbiodiniaceae phylogeny reconstructed by Decelle et al. (2018). A single ASV was nested within each clades: *Symbiodinium* (A.sym21),

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Breviolum (B.sym18), Foraminifera Clade G (G2.sym4), and *Halluxium* (H.sym12). Eight ASVs nested within *Cladocopium* (C.sym1, C.sym5, C.sym7, C.sym8, C.sym10, C.sym15, C.sym16, and C.sym17) and four ASVs within *Durusdinium* (D1.sym2, D1.sym6, D1.sym19, and D1.sym22).

Symbiodiniaceae distribution and diversity

The Venn diagrams show the overlap of the 16 ASVs identified as belonging to the Symbiodiniaceae by the site (Fig. 4A), by methodology, those are sample type (medium) and filter pore size (fraction) (Fig. 4B) (see also Supplemental file S6). The present/ absent sample table shows the Symbiodiniaceae proportion per subclade by site-sample type-filter pore size sample combination (Table 4 and Supplemental file S7). This table illustrates the common and unique subclades of Symbiodiniaceae. Three subclades were considerably common (C.sym1, D1.sym2, and G2.sym4), while the remaining subclades were unique (Table 4). The unique subclades (< 11.11% of subclade presence in all samples) show site or sample type-specific. C.sym1 was the most common (77,78%) and it detected at much more sites-medium-fraction than D1.sym2 (44.44%) and G2.sym4 (33.33%). Concerning medium, sediment samples appear to yield more Symbiodiniaceae subclades than seawater (12 vs. 7 subclades), with nine unique ASVs found in the sediment medium.

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Based on Symbiodiniaceae relative abundance composition appears that *Cladocopium* is the most dominant (Fig. 5). In general, Symbiodiniaceae of Lombok reef water characterized low alpha diversity and high beta diversity (Fig. 6). However, comparison analysis of Symbiodiniaceae abundances, observed features, and diversity does not show a significant difference between locations, media, and fractions (see Supplemental file S8).

DISCUSSION

The results illustrate the potential of eDNA to detect Symbiodiniaceae. The eDNA of Symbiodiniaceae can come from sources including free-living Symbiodiniaceae (Hirose et al., 2008; Littman, van Oppen & Willis, 2008) and Symbiodiniaceae living in symbiosis with various host organisms (Freudenthal, 1962; LaJeunesse et al., 2010, 2018; Pochon & Gates, 2010; Pochon, Putnam & Gates, 2014). In addition, these eDNA sources could came from within and outside the sample site (Goldberg et al. 2016). The aquatic and sediment environment contains Symbiodiniacean DNA released by prey organisms in their feces, through the shedding of host cells (Rees et al., 2014; Grupstra et al., 2021).

Biomolecular studies of Symbiodiniaceae have long been used the SSU 18S rRNA gene primer set (Rowan & Powers, 1991; Loh, Cowlishaw & Wilson, 2006). The use of hypervariable regions V4 and V9 isolated and amplified by the SSU 18S rRNA gene universal primer has succeeded in detecting and identifying Symbiodiniaceae from water samples (Stoeck et al.,

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Deleted: C.sym1 was detected at all sites, whereas D1.sym2 was found in all medium/fraction combinations. With respect to medium, sediment samples appear to yield more specialist OTUs than seawater (9 vs. 4 OTUs), with eight OTUs found in the sediment medium/0.4–12 µm pore size fraction combination.

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2010). This study used the same V9-SSU 18S rRNA gene primer set as used for oceanic planktonic Symbiodiniaceae by the Ocean TARA Expedition. The substitutions in the hypervariable terminal loop region amplified by this primer enable Symbiodiniaceae genera and subclades to be distinguished (Decelle et al., 2018). The use of other primers such as ITS, LSU 28S, and chloroplast primers can provide higher taxonomic resolution for the Symbiodiniaceae (Venera-Ponton et al., 2010; Takabayashi et al., 2012; Arif et al., 2014). Nevertheless, this study succeeded in detecting and identifying Symbiodiniaceae to the genus level.

Nonetheless , the use of universal eukaryote primers with eDNA samples can reveal information on a richer diversity of marine life, which can in some cases help to compensate for the high cost of using Next Generation Sequencing (Smart et al., 2016; Bálint et al., 2018). On the other hand, universal primers allow us to look at the system broadly and complete more than a single study from the same data (Madduppa et al., 2021).

As far as could be determined, this appears to be the first study of Symbiodiniaceae using eDNA in Indonesia and the Southeast Asia region more generally. Compared to previous studies in the Southeast Asia region, this study found a greater diversity of genera (see Table 5). These demonstrate the one advantage provided by the eDNA approach in water and sediment samples because most other studies focused on coral-associated symbionts, thus do not detect free-living genera or genera associated with less-studied hosts, such as foraminiferans.

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Comment [5]: Reviewer2:
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Response:
Thank you for your suggestion.

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Previous researches in the Southeast Asia region have isolated Symbiodiniaceae from scleractinian stone corals, sea slugs, giant clams and other bivalves, sea anemones, sponges, zoantharians, antipatharian black corals, and *Heliopora* blue corals. At least there have been discovered seven Symbiodiniaceae genere in Southeast Asia to date. The identification and characterize genetic diversity method of these genera used various primers such as nuclear primers (SSU 18S rRNA, LSU 28S rRNA, ITS1 rRNA, ITS2 rRNA, and microsatellite), mitochondrial organelle primers (mt COI, mt COB, and mt 16S), and chloroplast primers (psbAncr). Besides this also a range of molecular techniques such as Single Stranded Conformational Polymorphism (SSCP), Restriction Fragment Length Polymorphism (RFLP), and Denaturing Gradient Gel Electrophoresis (DGGE) (see Table 5). There was no reports about the genus *Effrenium* and clade I in Southeast Asia. However, clade E (AF238261.1) in our phylogeny (Fig. 3) had been assigned to clade D1 by Kimes et al. (2013). *E. voratum* is the only species from *Effrenium* described to date and only found in temperate waters (Jeong et al., 2014). LaJeunesse et al. (2012) predicted that the Southeast Asia region might have a higher diversity of Symbiodiniaceae spesies than other regions in the world. Together with the findings this of study, we support the prediction and therefore need to explore further other under-sampled coral reef areas in Indonesia.

The detected Symbiodiniaceae in this study sites are probably coral endosymbionts. *Symbiodinium, Breviolum, Cladocopium,* and *Durusdinium* are the main coral endosymbiont

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Comment [6]: Reviewer1:
Provide additional details to compare these other studies in Table 5 to the current study. What samples were analyzed, what primers/methods were used, what clades were detected?
Response:
We have mentioned that various hosts where Symbiodiniaceae are isolated, such as scleractinian stone corals, sea slugs, giant clams, other bivalves, sea anemones, sponges, zoantharians, antipatharian black corals, and Heliopora blue corals (see on line 313-314). We have also mentioned that there have been seven Symbiodiniaceae genera found. We also have added primers/identification methods in the text. Thank you for your suggestion.

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genera, while *Fugacium* and *Gerakladium* are rare endosymbionts in corals (LaJeunesse et al., 2010; Rouzé et al., 2017). The main coral endosymbiont, especially in Indo-Pacific, are *Cladocopium* and *Durusdinium*, whereas members *Symbiodinium* and *Brevolium* are more common in corals in the Caribbean (cBaker, 2003; LaJeunesse et al., 2004, 2010; LaJeunesse, 2005; Stat & Gates, 2011). Member *Cladocopium* (e.g., ITS2 subclade C1) function, in general, has higher rates of carbon fixation, provides a higher fitness benefit, translocates higher amounts of carbon to host corals, and affects host coral growth rate positively. In contrast, *Durusdinium* tends to be opportunistic even though it can help corals to survive or quickly recover from bleaching when sea surface temperature spikes occur (Stat, Morris & Gates, 2008; Stat & Gates, 2011; Lesser, Stat & Gates, 2013; Bay et al., 2016).

The current study detected three common subclades, namely C.sym1, D1.sym2, and G2.sym4. Likely, they show a feature of generalist Symbiodiniaceae type (see LaJeunesse et al., 2004). BLAST results showed that C.Sym1 was similar to *C. goreaui* (99.24%), formerly clade C type C1, is a generalist Symbiodiniaceae found in many coral hosts in the Great Barrier Reef (LaJeunesse, 2005; Bongaerts et al., 2015). D1.sym2 is strongly suspected to be *D. trenchii* because there was a 100% sequence similarity for the molecular marker used. *D. trenchii* is a Symbiodiniaceae which plays a role in increasing the capacity of corals to face bleaching stress (Stat & Gates, 2011). Previous studies have suggested the importance of a minimum density of *D. trenchii* as a minority component alongside a dominant endosymbiont from the genus

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Comment [7]: Reviewer1:
Not clear what is meant by a “balanced” symbiosis.
Response:
As endosymbiont, member Cladocopium (e.g., ITS2 subclade C1) generally have higher
rates of carbon fixation and provide a greater fitness benefit, translocate higher amounts of carbon to the host compared to members D (see Stat, Morris & Gates, 2008; Stat & Gates, 2011; Lesser, Stat & Gates, 2013). We have fixed it into the text (line 335-338).

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Cladocopium in the Symbiodiniaceae community within a coral colony (Bay et al., 2016). However, Swain et al. (2017) found that each genus within the Symbiodiniaceae has the potential for heat-resistant species or variants. For example, *C. thermophilum* is a thermo-tolerant variant of *Cladocopium* type C3 (Hume et al., 2015).

The detection of *Halluxium* in this study is interesting because it is the first to record in the Southeast Asia region. To date, studies found *Halluxium* in Guam, Heron Island (Great Barrier Reef, Australia), and the Caribbean (Pochon, LaJeunesse & Pawlowski, 2004; Pochon et al., 2007; Nitschke et al., 2020). This genus and the Clade I, in general, are foraminifera-specific endosymbionts. In contrast, previous studies never found *Breviolum,* or *Effrenium* species living as foraminifera endosymbionts (Pochon & Pawlowski, 2006; Pochon & Gates, 2010).

This study fully resolved the ASV of subclade G2.sym4 within the Foraminifera Clade G (formerly clade G type G2). This genus can be isolated from the Foraminifera, particularly in the subfamily Soritinae (Pochon et al., 2007). Bo et al. (2011) has also isolated a subclade close to type G2 from Indonesian octocorals. Foraminifera Clade G is a common endosymbiontic Symbiodiniaceae in sponges, such as in the bioeroding sponge (*Cliona orientalis*) in Australia (Schönberg & Loh, 2005; Ramsby et al., 2017). However, G2.sym4 appears to be a common type also found in the sediment samples. It indicates that this subclade may be an endosymbiont of benthic foraminifera. Previous studies showed that foraminifera communities around Lombok are diverse and widely distributed and present on the seabed in shallow coastal waters around the

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island (Auliaherliaty, Dewi & Priohandono, 2004; Natsir, 2009, 2010; Dewi et al., 2012). However, there are no published studies of foraminifera endosymbiotic Symbiodiniaceae in Indonesia.

The findings of more Symbiodiniaceae subclades in sediment than in seawater indicates potential benthic Symbiodiniaceae. These Symbiodiniaceae can have important implications for the coral reef ecosystems of Lombok. Previous studies found that the benthic sediment can be a source of free-living Symbiodiniaceae (Hirose et al., 2008; Littman, van Oppen & Willis, 2008; Fujise et al., 2021). The free-living Symbiodiniaceae can keep permanently or transiently (Yamashita & Koike, 2013). Corals regularly expel some of their endosymbionts into the seawater column (Fujise et al., 2014), most of which are deposited in sediments. Corallivorous, detrivorous, and herbivorous fishes can also contribute to the release and distribution of free-living Symbiodiniaceae in their habitat through their feces (Castro-Sanguino & Sánchez, 2012; Grupstra et al., 2021). The availability of such Symbiodiniaceae in the environment is essential. During the larval and/or recruitment time, most corals obtain Symbiodiniaceae horizontally from the nearby environment (Coffroth et al., 2006; Fujise et al., 2021). Their presence can also influence juvenile coral survival (Suzuki et al., 2013).

In this study, 13 of the 16 subclades were specialist type Symbiodiniaceae, indicating that these Symbiodiniaceae may have originated from local sources. Environmental genetic material is subject to degradation (Barnes & Turner, 2016) so that the concentration tends to center

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around the source. Therefore, eDNA tends to be representative of the local biotic material genetic. Shinzato et al. (2018) showed the feasibility of nearby coral species and their symbiotic algae detection using eDNA so that it might be possible to use it to monitor coral ecosystem health. However, interpreting such data need care as there are some issues regarding possible sources of eDNA from outside the sample site due to biological factors and human activities (Goldberg et al. 2016). There are some limitations of the eDNA method, such as the dependence on the presence and concentration of eDNA in the water sample, capture efficacy, extraction efficacy, sample interference (e.g., inhibition), and assay sensitivity (see Goldberg et al., 2016). Seawater eDNA samples can degrade beyond the detection threshold within one day to weeks (Dejean et al., 2011; Thomsen et al., 2012). Water quality conditions tend to increase the eDNA degradation rate, such as higher temperatures, neutral pH, moderately high UV-B (Strickler, Fremier & Goldberg, 2014). However, the degradation rate of eDNA in aquatic environments is different from that in sediments. The nature and proportion of minerals, organic substances, and charged particles that can adsorb eDNA fragments influence the rate of eDNA degradation in sediments and protect them from further destruction. The current study showed that the degradation rate of eDNA is about 57 times slower than in seawater (Torti, Lever & Jørgensen, 2015; Turner, Uy & Everhart, 2015; Sakata et al., 2020). After all, there is still little information regarding factors that influence the rate of symbiont DNA shed by coral reef taxa and maintained in the water column over spatial scales.

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CONCLUSION

This study demonstrates that eDNA surveys can describe the potential diversity of Symbiodiniaceae in the reefs around Lombok and identify six genera of Symbiodiniaceae. The findings indicate higher sensitivity compared to traditional methods. The presence of unique Symbiodiniaceae indicates that the eDNA originated close to the sampling site and supports the application of eDNA for monitoring local waters. However, there is a need for further confirmation through isolation from a variety of sources (including likely hosts) and microscopic observations to strengthen the evidence for local eDNA sources.

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ACKNOWLEDGEMENTS

The authors thank all staff and research colleagues who are members of the Center for Collaborative Research ANBIOCORE (Animal Biotechnology and Coral Reef Fisheries), IPB, the URI research team for their assistance during the research for technical laboratory, and the Laboratory of Marine Biodiversity and Biosystematics. The authors are also grateful to Prof. Dr. Ir. Sudarsono, M.Sc. for technical preparation to the manuscript draft.

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ADDITIONAL INFORMATION AND DECLARATIONS

Funding

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Grant Disclosures

BPPDN scholarship and USAID through the Sustainable Higher Education Research Alliances (SHERA) Program – Centre for Collaborative Research on Animal Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE) of IPB University.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Arief Pratomo conceived and designed the experiments, collected and processed eDNA samples, performed laboratory work and analysed the data, prepared figures and tables, authored or reviewed drafts of the paper, and approved the final draft.

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- Dietriech Geoffrey Bengen conceived the research idea, reviewed drafts of the paper, and approved the final draft.
- Neviaty Putri Zamani conceived the research idea, reviewed drafts of the paper, and approved the final draft.
- Christopher Lane conceived the research idea, reviewed drafts of the paper, and approved the final draft.
- Erin Borbee performed laboratory work and analysed the data, reviewed drafts of the paper, and approved the final draft.
- Austin T Humphries conceived the research idea, reviewed drafts of the paper, and approved the final draft.
- Hawis Madduppa contributed reagents/materials/analysis tools, conceived and designed the experiments, conceived the research idea, authored or reviewed drafts of the paper, and approved the final draft.

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Field Study Permissions

eDNA seawater sampling in this study was permitted within the framework of the United States Agency for International Development—Sustainable Higher Education Research Alliances (USAID-SHERA) program through the Centre for Collaborative Research Animal

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Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE) of IPB University, award no. AID-497-A-16-00004.

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Data Availability

The Fastq sequence raw data that supports the findings of this study are available at the public storage with following linkhttps://www.ncbi.nlm.nih.gov/sra/PRJNA768103 and Putative Symbiodiniaceae ASV sequence Data: https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP339775. Relevant analysis Code for this research analyses is available at https://github.com/arief2021/Symbio_Qiime2.git.

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Figure 1 Map of the research sites around Lombok Island, Indonesia. (A) West Lombok. (B) North Lombok. (C) East Lombok.

Figure 2 Proportion of Eukaryote taxa. Based on the total ASVs of taxon level 4 out of 15 taxon levels according to the SILVA database (https://www.arb-silva.de/).

Figure 3 Phylogenetic tree based on the V9-18S rRNA gene Maximum Likelihood of families in the Order Suessiales (A) and the Family Symbiodiniaceae (B). (A) The phylogeny of families in the Order Suessiales. ASVs from this study (OTU.sym1–OTU.sym22) are shown in bold black font, and the branch support values shown in red font represent the multi scores of SH-alrt/lby/Bayes/boot. Top: Motile stage of *Symbiodinium natans* and coccoid form of Symbiodiniaceae (source: LaJeunesse, 2020), Middle: Ventral view of *Borghiella dodgei* (source: Pandeirada, Craveiro & Calado, 2013), Bottom: Ventral view of *Polarella glacialis* (source: Montresor et al., 2003). (B) The phylogeny of genera in the Family Symbiodiniaceae. ASVs are shown in bold red, and red circles represent branch support scores >50 in SH-alrt. Phylogenetic reconstruction was performed in IQ-TREE and visualized with iToL (https://itol.embl.de/).

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Figure 4 Venn diagram of Symbiodiniaceae subclades around Lombok by: (A) coastal area and (B) method (sample type-filter pore size combination). For a more detailed explanation of this figure, see Supplemental File S6. Sample labels: sea = seawater sample; sed = sediment sample; _0.4 and _12 indicate the pore size of the filter (in µm).

Figure 5 Composition of the relative abundance of Symbiodiniaceae communities across different sites, sample types, and fractions. Relative abundance based on total presence of ASV frequencies. Bar graphs represent the total percent abundance of Symbiodiniaceae detected from all samples. Sample labels: sea = seawater sample; sed = sediment sample; 0.4-12 µm and >12 indicate the pore-size of the filter (in µm) sample.

Figure 6. **Total diversity of Symbiodiniaceae in coral waters around Lombok Island: (A) alpha diversity and (B) beta diversity.** Alpha diversity is indicated with Shannon Index and beta diversity with Bray-Curtis (BC) Dissimilarity. Boxplots display the median as the midline, the upper and lower quartiles as the top and bottom lines of the boxes, respectively. Crossing symbols indicate mean, and circles denote outliers.

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Figure 1

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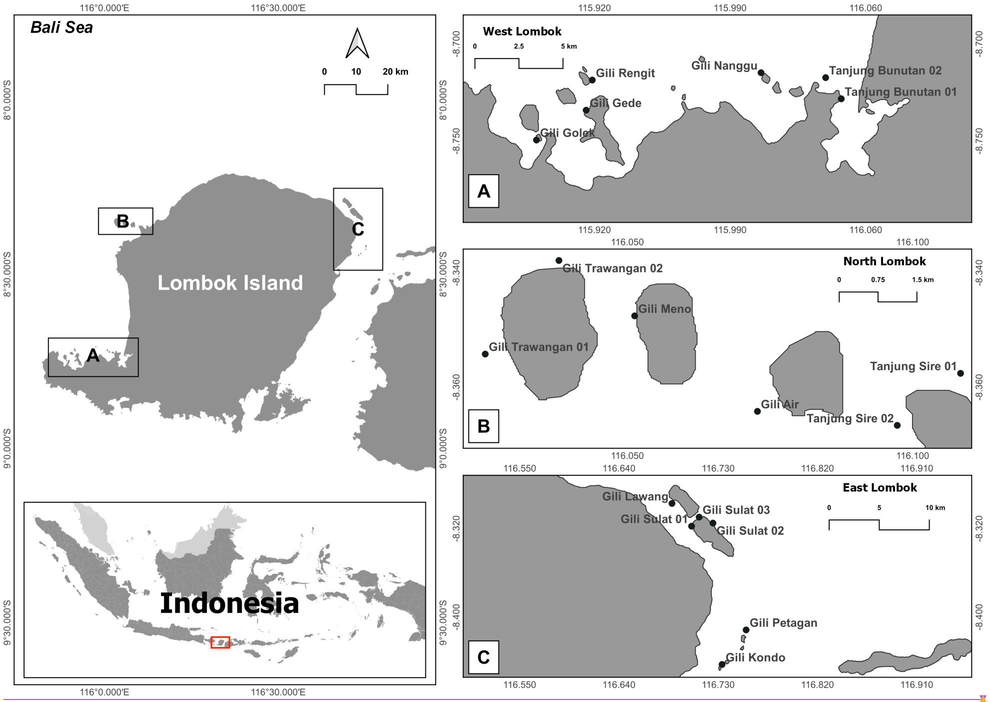

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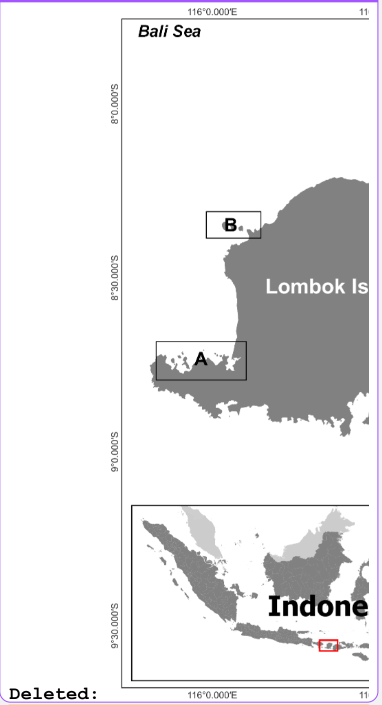

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Figure 2

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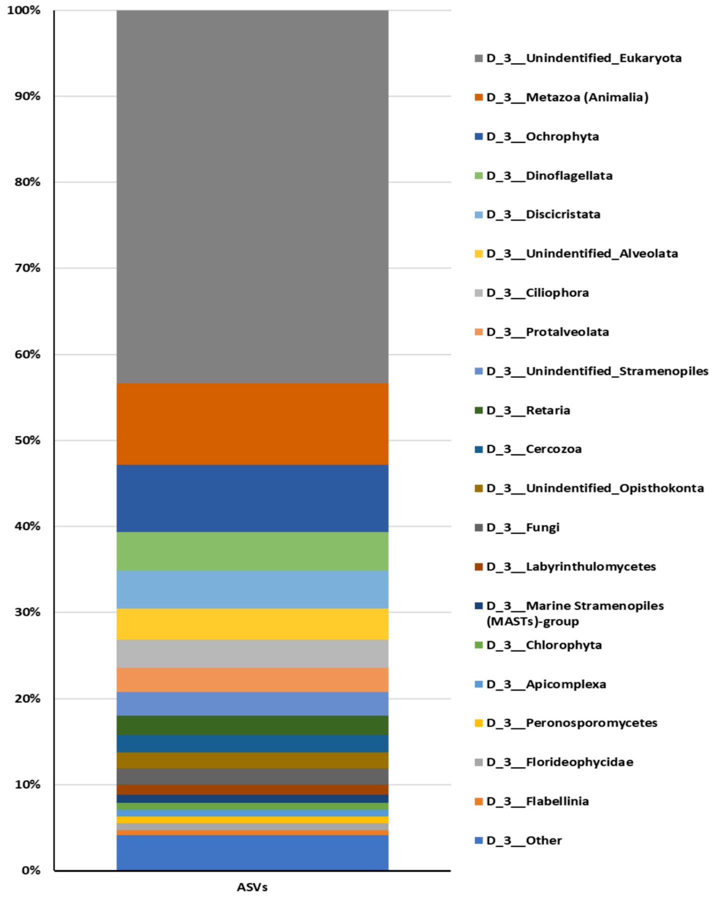

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Figure 3

Figure 3 **Phylogenetic tree based on the V9-18S rRNA gene Maximum Likelihood of the Order Suessiales (A) and the Family Symbiodiniaceae (B).** (A) The phylogeny of families in the Order Suessiales. ASVs from this study (OTU.sym1–OTU.sym22) are shown in bold black font, and the branch support values shown in red font represent the multi scores of SH-alrt/lbt/abayes/ufboot. Top: Motile stage of *Symbiodinium natans* and coccoid form of Symbiodiniaceae (source: LaJeunesse, 2020), Middle: Ventral view of *Borghiella dodgei* (source: Pandeirada, Craveiro & Calado, 2013), Bottom: Ventral view of *Polarella glacialis* (source: Montresor et al., 2003). (B) The phylogeny of genera in the Family Symbiodiniaceae. ASVs are shown in bold red, and red circles represent branch support scores >50 in SH-alrt. Phylogenetic reconstruction was performed in IQ-TREE and visualized with iToL (https://itol.embl.de/).

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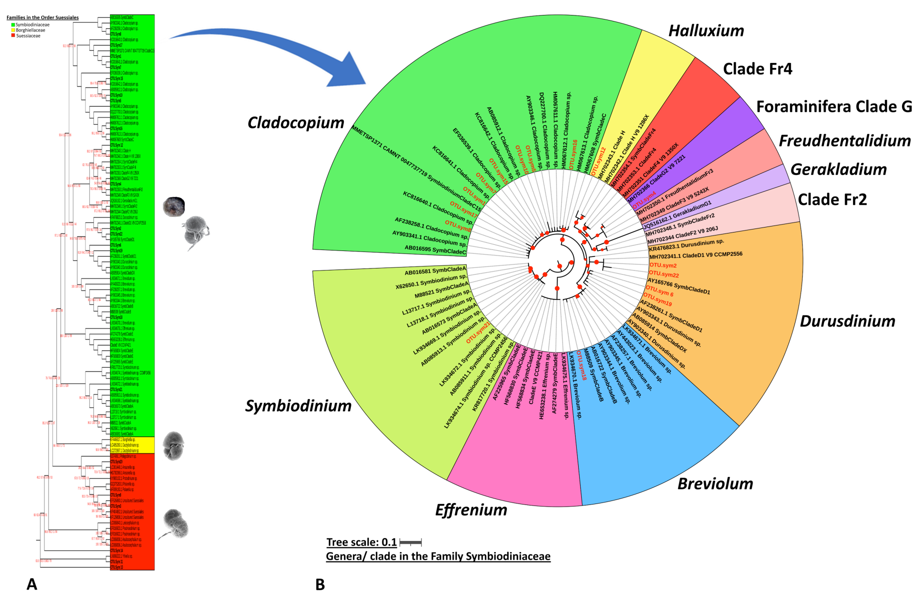

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Figure 4

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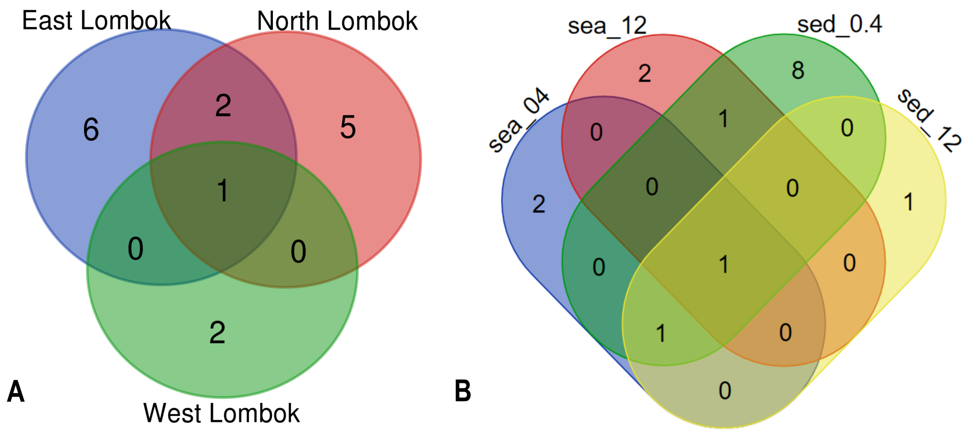

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Figure 5

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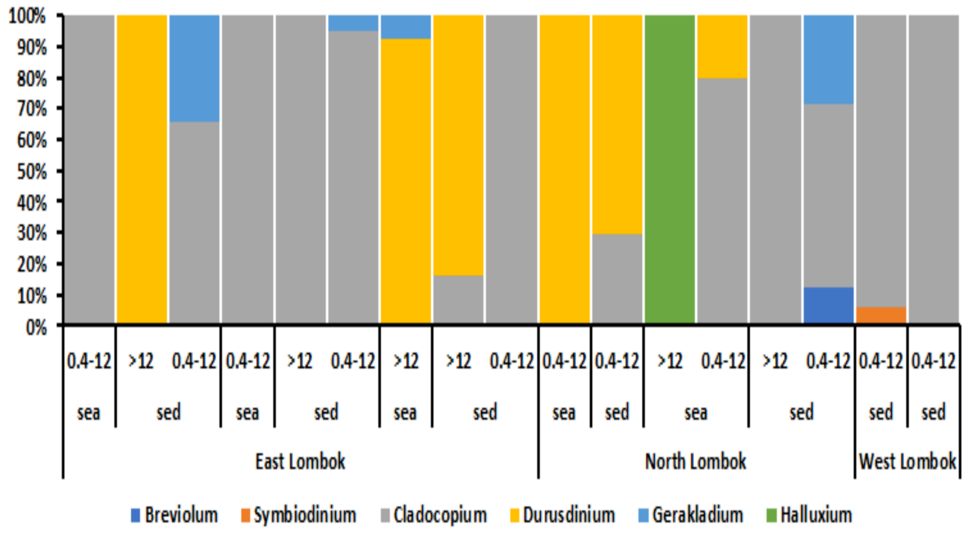

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Figure 6

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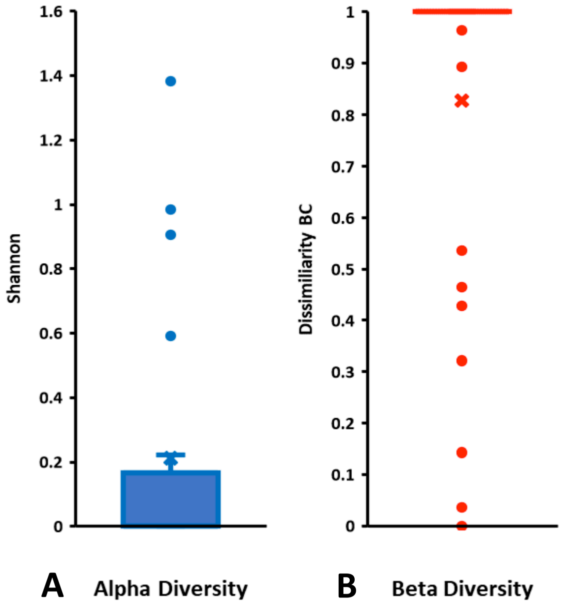

Table 1

Table 1 **Coordinates sampling stations around Lombok Island.**

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Coastal area	**Station**	**Date**	**Depth (m)***	**Position**	
				South	**East**
East Lombok	Gili Sulat 01	5 July 2018	< 1	08°19.069'	116°42.355'
	Gili Lawang	6 July 2018	1.2	08°17.833'	116°41.290'
	Gili Sulat 02	5 July 2018	> 10	08°18.900'	116°43.519'
	Gili Sulat 03	5 July 2018	< 1	08°18.574'	116°42.767'
	Gili Petagan	6 July 2018	2.8	08°24.698'	116°45.324'
	Gili Kondo	6 July 2018	< 1	08°26.572'	116°44.016'
North Lombok	Gili Trawangan 01	11 July 2018	8.46	08°21.253'	116°01.505'
	Gili Air	12 July 2018	< 1	08°21.854'	116°04.369'
	Gili Trawangan 02	11 July 2018	1.4	08°20.271'	116°02.280'
	Gili Meno	11 July 2018	> 10	08°20.852'	116°03.077'
	Tanjung Sire 01	12 July 2018	4.8	08°21.455'	116°06.506'
	Tanjung Sire 02	12 July 2018	8.3	08°22.001'	116°05.840'
West Lombok	Gili Nanggu	8 July 2018	< 1	08°42.887'	116°00.362'

Gili Rengit	9 July 2018	< 1	08°43.114'	115°55.135'
Gili Golek	9 July 2018	< 1	08°44.967'	115°53.405'
Gili Gede	9 July 2018	< 1	08°44.045'	115°54.945'
Tanjung Bunutan 01	8 July 2018	> 10	08°43.693'	116°02.848'
Tanjung Bunutan 02	8 July 2018	> 10	08°43.039'	116°02.363'

Notes:

* In lowest low water level (LLWL) based on Hydrographic and Oceanographic Center, The Indonesian Navy (2007) and mean tidal range is 187 cm.

Table 2

Table 2 **Successfully amplified eDNA samples by sample type and filter pore size.** EB356 – EB396 are the sample codes; n.a. (not available) indicates the eDNA samples were not successfully amplified; red font indicates Symbiodiniaceae were detected.

Location	**Station**	**Seawater fraction**		**Sediment fraction**	
		0.4 - 12 µm	**>12 µm**	**0.4 - 12 µm**	**>12 µm**
East Lombok	Gili Sulat 1	n.a.	EB356	EB357	EB358
	Gili Lawang	EB367	EB368	EB369	EB370
	Gili Sulat 2	EB359	EB360	EB361	EB362
	Gili Sulat 3	EB363	EB364	EB365	EB366
	Gili Petagan	n.a.	EB371	EB372	EB373
	Gili Kondo	n.a.	EB374	EB375	EB376
West Lombok	Gili Nanggu	n.a.	n.a.	EB377	n.a.
	Gili Rengit	n.a.	n.a.	EB379	n.a.
	Gili Golek	n.a.	n.a.	EB380	EB381
	Gili Gede	EB382	n.a.	EB383	n.a.

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	Bunutan 1	n.a.	n.a.	EB378	n.a.
	Bunutan 2	n.a.	n.a.	n.a.	n.a.
North Lombok	Gili Trawangan 1	EB384	EB385	EB386	EB387
	Gili Air	EB396	n.a.	n.a.	n.a.
	Gili Trawangan 2	EB388	EB389	EB390	EB391
	Gili Meno	EB392	EB393	EB394	EB395
	Tanjung Sire 1	n.a.	n.a.	n.a.	n.a.
	Tanjung Sire 2	n.a.	n.a.	n.a.	n.a.