Diversity and distribution of Symbiodiniaceae detected on coral reefs of Lombok, Indonesia using environmental DNA metabarcoding

Arief Pratomo [1, 2], **Dietriech G Bengen** [2], **Neviaty P Zamani** [2], **Christopher Lane** [3], **Austin T Humphries** [4], **Erin Borbee** [3], **Beginer Subhan** [Corresp., 2], **Hawis Madduppa** [2, 5]

[1] Raja Ali Haji Maritime University, Tanjungpinang, Indonesia

[2] Department of Marine Science and Technology, Institut Pertanian Bogor, Bogor, Indonesia

[3] Department of Biological Sciences, University of Rhode Island, Rhode Island, United States

[4] Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Rhode Island, United States

[5] Oceanogen Research Center, Bogor, Indonesia

Corresponding Author: Beginer Subhan
Email address: beginersubhan@apps.ipb.ac.id

Background: Dinoflagellates of family Symbiodiniaceae are important to coral reef ecosystems because of their contribution to coral health and growth ; however, only a few studies have investigated the function and distribution of Symbiodiniaceae in Indonesia . Understanding the distribution of different kinds of Symbiodiniaceae can improve forecasting of future responses of various coral reef systems to climate change. This study aimed to determine the diversity of Symbiodiniaceae around Lombok using environmental DNA (eDNA).

Methods: Seawater and sediment samples were collected from 18 locations and filtered to obtain fractions of 0.4–12 and >12 µm. After extraction, molecular barcoding polymerase chain reaction was conducted to amplify 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. We compared results across sampling sites, sample types (seawater/sediment), and filter pore sizes (fraction).

Results: Phylogenetic analyses resolved the amplicon sequence variants into 16 subclades comprising six Symbiodiniaceae genera (or genera-equivalent clades) as follows: *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, Foraminifera Clade G , and *Halluxium*. Comparative analyses showed that the three distinct lineages within *Cladocopium*, *Durusdinium*, and *Foraminifera Clade G* were the most common . Most of the recovered sequences appeared to be distinctive of different sampling locations, supporting the possibility that eDNA may resolve regional and local differences among Symbiodiniaceae genera and species.

Conclusions: eDNA survey s offer a rapid proxy for evaluating Symbiodiniaceae species on coral reefs and are a potentially useful approach to revealing diversity and relative ecological dominance of certain Symbiodiniaceae organisms. Moreover, Symbiodiniaceae eDNA analysis shows potential in monitoring the local and regional stability of coral–algal mutualisms.

Diversity and distribution of Symbiodiniaceae detected on coral reefs of Lombok, Indonesia using environmental DNA metabarcoding

Arief Pratomo[1,2], Dietriech Geoffrey Bengen[1], Neviaty Putri Zamani[1], Christopher E. Lane[3], Austin T. Humphries[4], Erin Borbee[3], Beginer Subhan[1*], Hawis Madduppa[1,5^]

[1]Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, IPB University, Bogor 16680, Indonesia

[2]Marine Science Study Program, Faculty of Marine Science and Fisheries, Raja Ali Haji Maritime University, Tanjungpinang, 29115, Indonesia

[3]Department of Biological Sciences, University of Rhode Island, 120 Flagg Rd Kingston, Rhode Island 02881 USA

[4]Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, 9 East Alumni Avenue, Kingston, Rhode Island 02881 USA

[5]Oceanogen Environmental Biotechnology Laboklinikum, Bogor, 16112, Indonesia

^Deceased

Corresponding Author: beginersubhan@apps.ipb.ac.id

Abstract

Background Dinoflagellates of family Symbiodiniaceae are important to coral reef ecosystems because of their contribution to coral health and growth; however, only a few studies have investigated the function and distribution of Symbiodiniaceae in Indonesia. Understanding the distribution of different kinds of Symbiodiniaceae can improve forecasting of future responses of various coral reef systems to climate change. This study aimed to determine the diversity of Symbiodiniaceae around Lombok using environmental DNA (eDNA).

Methods Seawater and sediment samples were collected from 18 locations and filtered to obtain fractions of 0.4–12 and >12 μm. After extraction, molecular barcoding polymerase chain reaction was conducted to amplify 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. We compared results across sampling sites, sample types (seawater/sediment), and filter pore sizes (fraction).

Results Phylogenetic analyses resolved the amplicon sequence variants into 16 subclades comprising six Symbiodiniaceae genera (or genera-equivalent clades) as follows: *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, Foraminifera Clade G, and *Halluxium*. Comparative analyses showed that the three distinct lineages within *Cladocopium*, *Durusdinium*, and *Foraminifera Clade G* were the most common. Most of the recovered sequences appeared to be

distinctive of different sampling locations, supporting the possibility that eDNA may resolve regional and local differences among Symbiodiniaceae genera and species.

Conclusions eDNA surveys offer a rapid proxy for evaluating Symbiodiniaceae species on coral reefs and are a potentially useful approach to revealing diversity and relative ecological dominance of certain Symbiodiniaceae organisms. Moreover, Symbiodiniaceae eDNA analysis shows potential in monitoring the local and regional stability of coral–algal mutualisms.

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

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

INTRODUCTION

Symbiodiniaceae, also known as zooxanthellae, play vital roles within their coral hosts, such as providing energy, absorbing residual metabolites, and promoting growth (Davy, Allemand & Weis, 2012; Purnomo, 2014). These symbionts also contribute to the adaptability and resilience of corals to environmental change, especially 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 stress (Abrego et al., 2008; LaJeunesse et al., 2010; Stat & Gates, 2011; Cunning, Silverstein & Baker, 2015; Bourne, Morrow & Webster, 2016; Hoadley et al., 2019). Therefore, understanding the potential diversity of Symbiodiniaceae is necessary in forecasting the future of coral reef ecosystems in different regions under a rapidly changing climate.

Endosymbiotic dinoflagellates of family Symbiodiniaceae are extremely prevalent in coral reef ecosystems. Symbiodiniaceae engage in mutualistic relationships with various invertebrates, including scleractinian corals, octocorals, anemones, jellyfishes, mollusks, sponges, flatworms, and foraminifera (Pochon et al., 2001; LaJeunesse et al., 2010, 2018; LaJeunesse, Pochon, Putnam & Gates, 2014). A number of Symbiodiniaceae live as aquatic plankton and benthic periphyton, and some are associated with macroalgae and seagrasses (Venera-Ponton et al., 2010; Takabayashi et al., 2012; Fujise et al., 2021). To date, 11 named

genera have been classified as members of 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, there are 16 distinct lineages, with Foraminifera Clade G, Clade Fr2, Clade Fr4, Clade I, and Clade J representing the undescribed genera (LaJeunesse et al., 2018; Yorifuji et al., 2021).

Indonesia is a part of the Coral Triangle (Veron et al., 2009; Gelis et al., 2021), and coral reef ecosystems are a valuable economic resource for coastal communities across the archipelago; however, data on the diversity of Indonesian Symbiodiniaceae are still limited (Loh et al., 2006, Bo et al., 2011, Purnomo 2014, DeBoer et al., 2012). Previous studies about Symbiodiniaceae from areas in the region such as the South China Sea, Thailand, Singapore, Palau, the Philippines, and Timor-Leste, only focused on Symbiodiniaceae populations within their host organisms. Some of the Symbiodiniaceae genera in these reports include *Symbiodinium, Breviolum, Cladocopium, Durusdinium, Gerakladium*, and *Fugacium* (Fabricius et al., 2004; Loh, Cowlishaw & Wilson, 2006; (Reimer & Todd, 2009); LaJeunesse et al., 2010; Taguba, Sotto & Geraldino, 2016; Tong et al., 2018; Brian, Davy & Wilkinson, 2019). However, little is known about Symbiodiniaceae within Indonesian waters, which is the most biodiverse mairne region in the world.

Symbiodiniaceae cannot be directly identified using conventional microscopy. The need for collection and isolation from multiple locations increases the difficulty of assessing this taxonomic group. Advances in the use of environmental DNA (eDNA) and multitaxon sequencing techniques (metabarcoding) have allowed the study of 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 ease of use, noninvasive nature, broad spatial scale, and cost effectiveness (Deiner et al., 2017).

This study aimed to develop a rapid proxy for estimating the diversity of Symbiodiniaceae in water and sediment samples from the coral reef ecosystems around Lombok Island in Indonesia using eDNA. We make comparisons across sampling sites, eDNA source (seawater/sediment), and filter pore sizes (fraction). A better understanding of the diversity and composition of Symbiodiniaceae in Indonesian coral reefs is important for conservation and management of marine ecosystems.

METHODS

Study sites

This 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

western part of Lombok Island is directly adjacent to Lombok Strait and its southern part is the Indian Ocean. The study areas were located 5–100 m from shore, with depths ranging 1–10 m and a mean tidal range of about 1.8 m. Samples were collected from 5th to 12th July 2018 (Table 1.).

eDNA sample collection

During the survey, eDNA seawater and sediment samples were collected by scuba diving from six reef stations within each coastal area (West Lombok, East Lombok, and North Lombok). Two samples (one seawater and one sediment) per station were collected per day from three stations, in total, 72 samples in Lombok (Fig. 1 and Table 1, 2). The distance between the sampling stations was at least 1500 m to avoid overlap. At each station, 4 L of seawater was collected from the water column (~2 m above the reef substrate) as well as a sediment sample (water + sediment in 1:1 ratio) in sterilized bottles. Before sampling, the bottle was rinsed with a 30% commercial bleach solution, followed by distilled water. The collected eDNA samples were stored in a cool box and brought to basecamp at Lombok Island as soon as possible (less than 12 hours). Each sample was filtered twice using a peristaltic pump (Fisher Scientific) through 47 mm diameter polycarbonate membrane filters (Sterlitech) with two different pore sizes: 12 μm first and then 0.4 μm. According to Turner et al. (2014), a combination of ≥ 0.2 μm filtration pore size and water volume enables optimal eDNA capture and maximize detection probability.

126 In addition, a large pore size is required to avoid clogging the filters. The sediment samples,
127 were shaken first and then filtered 1–2 minutes after shaking. Each filter was cut into two, and
128 each half was placed in a 1.5 mL vial prefilled with DNA Shield as a preservative. At the end of
129 all eDNA survey activities, all the samples were transported to the Marine Biodiversity and
130 Biosystematics Laboratory at Bogor Agricultural (IPB) University, Indonesia, via commercial
131 courier and then stored at −20 °C until DNA extraction.
132 eDNA seawater sampling in this study was permitted within the framework of the United
133 States Agency for International Development—Sustainable Higher Education Research
134 Alliances (USAID-SHERA) program through the Centre for Collaborative Research Animal
135 Biotechnology and Coral Reef Fisheries of IPB University, award no. AID-497-A-16-00004. The
136 field research permit was issued by IPB University Rector (Surat Tugas no. 403/IT3/KP/2019).
137 Permits for this research were issued by the Indonesian Ministry of Research and Technology to
138 EB (130/E5/E5.4/SIP/2019), CL (461/SIP/FRP/E5/Dit.KI/XII/2017), and AH
139 (455/SIP/FRP/E5/Dit.KI/XII/2017).
140
141 **DNA extraction, amplification, and sequencing**
142 The filtered eDNA samples were extracted and amplified at the Marine Biodiversity and
143 Systematic Laboratory of IPB University and sequenced at the University of Rhode Island (URI)
144 Genomics and Sequencing Center, United States of America. DNA was extracted from the 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 polymerase chain reaction (PCR) platform and prepared for 2×250 bp paired-end Illumina MiSeq sequencing (Illumina, San Diego, CA, United States). Amplification was conducted using 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), Illumina adapters, linker sequences, index, and pad (Kozich et al., 2013). The PCR profile used was as follows: 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, and a final extension at 72 °C for 5 min. Each 49 µL of PCR reaction comprised 25 µL of MyTM HS red mix (Bioline Ltd., London, UK), 1 µL of (10 µM) forward primer, 1 µL of (10 µM) reverse primer, and 1 µL of DNA template. The final volume was adjusted to 49 µL using ddH2O. 1x reaction was 0.2 µM. The PCR product was checked via the electrophoresis final master mix concentration in 1× reaction was 0.8×, and the final primer concentration in of 5 µL of aliquots on 1% agarose gel in 0.5X TBE buffer. Library preparation and sequencing were performed at URI. 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 as follows: initial denaturation at 95 °C for 3 min, followed by 9 cycles of 95 °C for 30 s and 55 °C for 30 s, and final extension at 72 °C for 5 min.

164 The presence and length (bp) of the PCR product or amplicon were tested by electrophoresis.
165 Successful amplicons were then purified using paramagnetic Kapa pure beads (bead-to-sample
166 volumetric ratio in 1.6:1). A Qubit fluorometer with Qubit dsDNA HS Assay reagent
167 (Invitrogen, California, US) was used to quantify all libraries. The prepared samples were
168 combined in equal concentrations and then pooled with a 20% denatured and diluted PhiX
169 Illumina control library. The final pooled library was sequenced on an Illumina MiSeq with the
170 MiSeq v2 500-cycle kit (Illumina, San Diego, CA, United States). After quality checking, only
171 41 out of 72 samples were found to be of sufficiently high quality for sequencing (Table 2). The
172 low quality of some libraries may be due to eDNA degradation during sample transport and
173 extraction.

174

175 **Data processing and bioinformatic analyses**

176 The obtained forward and reverse raw sequence data were converted to demultiplexed fastq files
177 (see additional information on data availability). The sequence read quality was checked using
178 FastQC v.0.11.8 (https://www.bioinformatics.babraham.ac.uk) at each analysis step. Cutadapt
179 v.1.18 (Martin, 2011) was used to trim the reverse and forward primer sequences and remove
180 short reads with lengths < 100 bp and low quality reads with a Phred Q score of < 20.
181 Qiime2.2019.10 pipeline (Caporaso et al., 2010; Bolyen et al., 2019) was employed for further
182 data processing. DADA2 v.2018.11.0 (Callahan et al., 2016) (via q2-dada2) was applied for

183 denoising, joining denoised paired-end reads, filtering out chimeric sequences and singletons,
184 and dereplicating sequences to produce amplicon sequence variants (ASVs). Owing to the high
185 quality of the sequences obtained after Cutadapt procedure, trimming and truncating were not
186 performed during DADA2 processing.
187
188 **ASV identification**
189 Symbiodiniaceae species were identified from the eDNA sequences by classifying all ASVs
190 (Supplemental File S1) using the q2-feature-classifier (Bokulich et al., 2018) classify-sklearn Fit-
191 Naïve Bayes taxonomy classifier against the 18S NR SILVA (release 123 Qiime compatible)
192 97% and 99% OTU reference sequences (https://www.arb-silva.de/download/archive/qiime/).
193 Stoeck et al. (2010) showed the differential increase in diversity detected when the V9 dataset is
194 clustered at 97%, 98%, 99%, and 100% sequence similarity for the minimum expected error rate.
195 Putative Symbiodiniaceae ASVs were then filtered from the obtained eukaryote taxonomy table
196 (Supplemental File S2) (Table 3) and then assessed using the NCBI BLAST routine by selecting
197 the best hit at >95% identity in the nr/nt database of NCBI (https://www.ncbi.nlm.nih.gov/,
198 accessed on 1/19/2020, version 2.11.0). The BLAST results (Supplemental File S3) were
199 evaluated, and reference sequences (accessions) were selected for further analyses. Additional
200 SSU 18S Symbiodiniaceae reference sequences (accessions) representing several families in the
201 order Suessiales, family Symbiodiniaceae were obtained from the NCBI database and the V9-

SSU 18S sequence reference database of TARA Ocean Expedition (Decelle et al., 2018) and Loh et al. (2006). The final compiled reference sequence database (Supplemental File S4) contained 82 sequences. These reference sequences and the putative Symbiodiniaceae ASVs from the samples were then aligned with MAFFT v.7 (Katoh & Standley, 2013) (via q2-alignment), followed by masking (Rajan, 2012). A phylogenetic tree representing the evolutionary relationships of Symbiodiniaceae members was constructed using the maximum likelihood approach in the IQ-TREE v.1.6.12 (Nguyen et al., 2015) (via q2-phylogeny) with 1000 bootstraps. These parameters were adopted to calculate the phylogenetic branch support scores from Shimodaira and Hasegawa approximate likelihood ratio test (SH-alrt) with local bootstraps (lbt), Bayesian (abayes), and ultrafast bootstraps (ufboot). Detailed explanations for these scores are provided in the IQ-TREE documentation (Minh et al., 2021). The best-fit substitution model TIM3 + F + R3 was chosen according to the Bayesian Information Criterion by ModelFinder applied in IQ-TREE (Kalyaanamoorthy et al., 2017). The Symbiodiniaceae taxonomic nomenclature was adopted from LaJeunesse et al. (2018). The term subclade was used instead of species because the 18S short eDNA sequence cannot be resolved to species-level for Symbiodiniaceae.

Statistical analyses

The relative abundance data for the putative Symbiodiniaceae taxa (Supplemental File S5) were from DADA2 results and used as input for Venn diagram and statistical analyses. Venn diagram analyses were performed using the online application in http://bioinformatics.psb.ugent.be/webtools/Venn/ to compare the Symbiodiniaceae individuals across different locations (coastal area), eDNA source (seawater and sediment samples), and fractions (filter pore size). We determined the most commonly distributed subclades and distinctive subclades to each location/station. Statistical analyses were used to compare Symbiodiniaceae abundance, diversity, and features observed across different sites, sample types, and fractions. All these statistical analyses were carried out on Qiime2.2019.10 pipeline (Caporaso et al., 2010; Bolyen et al., 2019). Alpha diversity (observed features and Shannon's entropy) and beta diversity (Bray–Curtis dissimilarity) were estimated using q2-diversity after the samples were rarefied (subsampled without replacement) to 28 sequences per sample. The comparison of all samples were grouped by location, eDNA source, and fraction to examine differences in abundance and alpha diversity employing the Kruskal–Wallis test (Kruskal & Wallis, 1952 and beta diversity applying the Permanova test (Anderson, 2001) using 9999 permutations.

RESULTS

238 **Obtained sequences, ASVs, and eukaryote classification**

239 From the 72 samples across 18 stations, DNA was successfully extracted from 41 samples at 16
240 stations, yielding a total of 3,168,655 raw sequences and about 30,205–240,604 sequences per
241 sample (Table 2 and Fig. S1). DADA2 yielded a total of 20,486 ASVs (Supplementary File S1).
242 The mean length of the obtained sequences was 127.81 ± 22.03 bp. The ASV classification
243 demonstrated the potential diversity of eukaryotes in the reef waters of Lombok Island.
244 According to the total ASVs classified to taxon level 4 from the SILVA database, the dominant
245 taxon was unclassified eukaryotes (43.35%), followed by Metazoa (9.47%), Ochrophyta
246 (7.83%), Dinoflagellates (4.5%), and Discicristata (4.4%) (Fig. 2).

247

248 **Symbiodiniaceae detection and classification**

249 Table 3 summarizes the results of Symbiodiniaceae classification performed using a Eukaryote
250 classifier (Supplemental File S2) and BLAST (Supplemental File S3) and phylogenetic analyses.
251 The probabilistic classifier detected and classified the Symbiodiniaceae taxa at the family level.
252 Twenty-two ASVs (named OTU.sym1 to OTU.sym22) were found to be putative
253 Symbiodiniaceae with confidence levels ranging 0.743–0.999 (Table 3). BLAST results
254 indicated that some ASVs were neither Symbiodiniaceae nor classified at the genus level. A
255 partial phylogenetic reconstruction of the families in order Suessiales was conducted using the

256 reference sequences obtained from the searched databases (Supplemental File S4) and the
257 putative Symbiodiniaceae ASV sequences from the study (Fig. 3A). Only 16 out of the 22 ASVs
258 were identified as members of the monophyletic group of the Symbiodiniaceae family clade on
259 the basis of the score of 100/100/1/99 for SH-alrt/lbt/abayes/ufboot. Three of the six remaining
260 ASVs were categorized in the clades representing genera in Family Suessiaceae, two ASVs were
261 in the *Yihiella* clade (OTU.sym11 and OTU.sym13), and one was in the *Ansanella* clade
262 (OTU.sym20). The remaining three ASVs were designated to the Suessiaceae family but were
263 not classified at the genus level (OTU.sym3, OTU.sym9, and OTU.sym14).
264 The Symbiodiniaceae family branch (Fig. 3B) comprised six clades, each representing
265 one genus with strong-to-moderate support (see the scores in Table 3 and Fig. 3). This
266 phylogenetic topology is concordant with the Symbiodiniaceae phylogeny reconstructed by
267 Decelle et al. (2018). One ASV was allocated to each of clades *Symbiodinium* (A.sym21),
268 *Breviolum* (B.sym18), Foraminifera Clade G (G2.sym4), and *Halluxium* (H.sym12). Eight ASVs
269 were designated to *Cladocopium* (C.sym1, C.sym5, C.sym7, C.sym8, C.sym10, C.sym15,
270 C.sym16, and C.sym17), and four ASVs allocated to Durusdinium (D1.sym2, D1.sym6,
271 D1.sym19, and D1.sym22).
272
273 **Symbiodiniaceae distribution and diversity**

Venn diagrams show the overlap of the 16 ASVs belonging to Symbiodiniaceae according to location (Fig. 4A) and sample type (media and fractions) (Fig. 4B) (see also Supplemental file S6). The presence/absence table shows the Symbiodiniaceae proportion per subclade by site–sample type–filter pore size combination (Table 4 and Supplemental file S7). This table illustrates the common and unique subclades of Symbiodiniaceae. The unique subclades were the sequences distinctive of sampling location, medium, and fraction. Three subclades were most common (C.sym1, D1.sym2, and G2.sym4), and the remaining subclades were unique (Table 4). The unique subclades (< 11.11% of subclade presence in all samples) showed site- or sample type-specificity. C.sym1 was the most common (77,78%) and was detected at more sites–media–fractions than D1.sym2 (44.44%) and G2.sym4 (33.33%). In term of medium, the sediment samples yielded more Symbiodiniaceae subclades than seawater (12 vs. 7 subclades), with nine unique ASVs found in the sediment medium.

On the basis of Symbiodiniaceae relative abundance, genus *Cladocopium* was the most dominant (Fig. 5). In general, the Symbiodiniaceae communities of Lombok were characterized with low alpha diversity and high beta diversity (Fig. 6). However, comparison of Symbiodiniaceae abundances, observed features, and diversity does not show 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 be obtained from different sources including free-living Symbiodiniaceae (Hirose et al., 2008; Littman, van Oppen & Willis, 2008) and Symbiodiniaceae living in symbioses with various host organisms (Freudenthal, 1962; Loh et al.,2006; Barneah et al., 2007; LaJeunesse et al., 2010, 2018; Pochon & Gates, 2010; DeBoer et al., 2012; Pochon, Putnam & Gates, 2014; Ramsby et al., 2017). Additionally, these eDNA sources could come from within and outside the sample site (Goldberg et al. 2016). Symbiodiniaceae DNA could be obtained from prey organism feces and through the shedding of host cells in the water and sediment (Rees et al., 2014; Grupstra et al., 2021).

The SSU 18S rRNA gene primer set has long been used in the biomolecular studies of Symbiodiniaceae (Rowan & Powers, 1991; Loh, Cowlishaw & Wilson, 2006). Hypervariable regions V4 and V9 isolated and then amplified by the SSU 18S rRNA gene universal primer were successful in detecting and identifying Symbiodiniaceae from water samples (Stoeck et al., 2010). This study used the same V9-SSU 18S rRNA gene primer set for oceanic planktonic Symbiodiniaceae by the Ocean TARA Expedition. The substitutions in the hypervariable terminal loop region amplified by this primer allowed us to distinguish Symbiodiniaceae genera and subclades (Decelle et al., 2018). Other primers such as ITS, LSU 28S, and chloroplast primers can be used to provide high taxonomic resolution for Symbiodiniaceae (Venera-Ponton

et al., 2010; Takabayashi et al., 2012; Arif et al., 2014). Nevertheless, this study succeeded in detecting and identifying Symbiodiniaceae at the genus level.

The use of universal eukaryote primers with eDNA samples can reveal information on the rich diversity of marine life and compensate for the high cost of next-generation sequencing (Smart et al., 2016; Bálint et al., 2018). Universal primers allow us to broadly look at the system and complete more than a single study using the same data (Madduppa et al., 2021). The lack of field blanks (non-reef sampling areas) and filter blanks (distilled water or sterile seawater samples), might influence our study results. Lack of control/blanks can lead to contamination of the eDNA source, or false-positive data. However, the comparitive analyses across the given samples allowed the evaluation of the possibility of exogenous and local eDNA sources. Moreover, the presence of contaminant DNAs was likely suppressed by rinsing the instruments (e.g., bottle samples and filtering tools) with bleach to make them as sterile as possible.

To the authors' knowledge, this work is the first study of Symbiodiniaceae using eDNA in Indonesia and Southeast Asia. Symbiodiniaceae in the Southeast Asia region have been identified from scleractinian stony corals, sea slugs, giant clams, and other bivalves, sea anemones, sponges, zoantharians, antipatharian black corals, and *Heliopora* blue corals. At least seven Symbiodiniaceae genera have been discovered in Southeast Asia (Table 5). Various primers, such as nuclear primers, mitochondrial organelle primers, and chloroplast primers, and a range of molecular techniques such as single stranded conformational polymorphism, restriction fragment

length polymorphism, and denaturing gradient gel electrophoresis, have been used in the identification and characterization of the genetic diversity of Symbiodiniaceae in the region but did not detect as many genera as the present study did (see Table 5). No report was found about the genus *Effrenium* and Clade I in Southeast Asia. However, clade E (AF238261.1) in our phylogeny (Fig. 3) was assigned to clade D1 by Kimes et al. (2013). *E. voratum* is the only species from *Effrenium* that was previously described and is 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 species than other regions in the world. Previous and current findings supports this prediction (Loh *et al*., 2006; Bo *et al*., 2011; DeBoer *et al*., 2012; Purnomo 2014). Therefore, other under-sampled coral reef areas in Indonesia should be further explored.

The detected Symbiodiniaceae in the study sites are probably coral endosymbionts. Some species of *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium* are the main coral endosymbiont genera, and species of *Fugacium* and *Gerakladium* are rare endosymbionts in corals (LaJeunesse et al., 2010; Rouzé et al., 2017). The main coral endosymbionts, especially in Indo-Pacific, are species of *Cladocopium* and *Durusdinium*; meanwhile members *Symbiodinium* and *Brevolium* are common in corals in the Caribbean (Baker, 2003; LaJeunesse et al., 2004, 2010; LaJeunesse, 2005; Stat & Gates, 2011). Many members of *Cladocopium* (e.g., ITS2 subclade C1) generally have high rates of carbon fixation, provide a high fitness benefit, translocate high amounts of carbon to host corals, and positively impact host coral growth rates.

By contrast, some species of *Durusdinium* tend to be opportunistic, even though they can help corals to survive or quickly recover from bleaching when sea surface temperatures rise (Stat, Morris & Gates, 2008; Stat & Gates, 2011; Lesser, Stat & Gates, 2013; Bay et al., 2016).

This study detected the three most common subclades, namely C.sym1, D1.sym2, and G2.sym4. These subclades may represent the most common species or types of Symbiodiniaceae. BLAST results showed that C.Sym1 was similar to *C. goreaui* (99.24%), formerly clade C type C1, which is a generalist Symbiodiniaceae found in many coral hosts in the Great Barrier Reef (LaJeunesse, 2005; Bongaerts et al., 2015). The sequence of D1.sym2 detected by BLAST has 100% sequence similarity with the molecular marker of *D. trenchii*, a Symbiodiniaceae species that increases the tolerance of corals to 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 *Cladocopium* in the Symbiodiniaceae community within a coral colony (Bay et al., 2016). However, Swain et al. (2017) found that each genus of Symbiodiniaceae has the potential for heat-resistant species or variants. For example, *C. thermophilum* is a thermotolerant variant of *Cladocopium* type C3 (Hume et al., 2015).

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 Subfamily Soritinae (Pochon et al., 2007). Bo et al. (2011) also isolated a subclade close to type

G2 from Indonesian octocorals. Foraminifera Clade G is a common endosymbiotic Symbiodiniaceae in sponges, such as bio-eroding sponge (*Cliona orientalis*) in Australia (Schönberg & Loh, 2005; Ramsby et al., 2017). However, G2.sym4 appears to be a common type and is also found in the sediment samples. Therefore, this subclade may be an endosymbiont of benthic foraminifera. Foraminifera communities around Lombok are diverse, widely distributed, and present in the seabed in shallow coastal waters around the island (Auliaherliaty, Dewi & Priohandono, 2004; Natsir, 2009, 2010; Dewi et al., 2012). However, no studies of foraminifera endosymbiotic Symbiodiniaceae in Indonesia have been published.

The detected *Halluxium* in this study is the first record in the Southeast Asia region. To date, *Halluxium* has only been found 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 Clade I are generally foraminifera-specific endosymbionts. Meanwhile, *Breviolum* or *Effrenium* species living as foraminifera endosymbionts have never been reported (Pochon & Pawlowski, 2006; Pochon & Gates, 2010).

The richer Symbiodiniaceae subclades in sediment than in seawater indicate the potential occurrence of benthic Symbiodiniaceae. These Symbiodiniaceae can have important implications for the coral reef ecosystems of Lombok. The benthic sediment can be a source of free-living Symbiodiniaceae that live outside the host (Hirose et al., 2008; Littman, van Oppen & Willis, 2008; Fujise et al., 2021). Some of these can (re-) establish stable host–algal mutualisms

(transient free-living), and others are true free-living, such as *E. voratum* (Yamashita & Koike, 2013; Jeong et al., 2014). Some transient free-living Symbiodiniaceae can come from expelled coral endosymbionts. Corals regularly expel some of their endosymbionts into the seawater column (Fujise et al., 2014), most of which are deposited in sediments. The other source of transient free-living Symbiodiniaceae is reef fishes. Corallivorous, detritivorous, and herbivorous fishes can contribute to the release and distribution of transient 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 larval stage and/or recruitment time, most corals horizontally obtain transient free-living Symbiodiniaceae from the nearby environment (Coffroth et al., 2006; Fujise et al., 2021). The presence of such Symbiodiniaceae can also influence juvenile coral survival (Suzuki et al., 2013).

This study found that 13 of the 16 subclades were distinctive of different sampling locations. These subclades may represent the species or types of Symbiodiniaceae originating from local sources. Environmental genetic materials are prone to degradation (Barnes & Turner, 2016), so they tend to accumulate around the source. Therefore, eDNA is representative of the local biotic genetic material. Shinzato et al. (2018) showed the feasibility of studying nearby coral species and their symbiotic algae detection using eDNA; therefore, it might also be used to monitor coral ecosystem health. However, such data must be carefully interpreted because of

some issues regarding the possible sources of eDNA from outside the sample site due to biological factors and human activities (Goldberg et al., 2016).

The eDNA method also has some limitations, 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 1 day to weeks (Dejean et al., 2011; Thomsen et al., 2012). Water quality conditions, such as high temperatures, neutral pH, and moderately high UV-B, tend to increase the eDNA degradation rate (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 adsorbing eDNA fragments influence the rate of eDNA degradation in sediments and protect them from further destruction. A previous study showed that the degradation rate of eDNA in sediment is about 57 times slower than that in seawater (Torti, Lever & Jørgensen, 2015; Turner, Uy & Everhart, 2015; Sakata et al., 2020). Limited information is available regarding the factors that influence the rate of symbiont DNA shed by coral reef taxa and maintained in the water column over spatial scales.

CONCLUSIONS

This study demonstrates that eDNA surveys can describe the potential diversity of Symbiodiniaceae in the reefs around Lombok. Six genera (or genera-equivalent clades) of Symbiodiniaceae were identified. eDNA survey has higher sensitivity than traditional methods and thus offer a rapid proxy for evaluating Symbiodiniaceae communities across different coral reefs. This approach can also be used to enhance the understanding of the diversity and relative ecological dominance of certain Symbiodiniaceae members. Moreover, the presence of distinctive Symbiodiniaceae individuals in different locations support the potential application of eDNA for monitoring the local and regional stability of coral–algal mutualisms. Further confirmation through isolation from a variety of sources (including possible hosts) and microscopic observations is warranted to strengthen the evidence for local eDNA sources.

ACKNOWLEDGEMENTS

This manuscript is dedicated to the memory of our dear colleague, friend, and dive buddy Dr. Hawis Madduppa. We will always remember your infectious smile, curiosity for the marine environment, and confident leadership. The authors thank all staff and research colleagues who are members of the Center for Collaborative Research ANBIOCORE (Animal Biotechnology and Coral Reef Fisheries), Bogor Agricultural University, the University of Rhode Island research team for their assistance during the research, 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.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was supported by the BPPDN scholarship to AP and USAID through the SHERA Program – Centre for Collaborative Research on Animal Biotechnology and Coral Reef Fisheries of IPB University to HM, CL, and AH.

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.

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 analyzed the data, prepared figures and tables, authored or reviewed drafts of the paper, and approved the final draft.
- 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 and designed the experiments, conceived the research idea, authored or reviewed drafts of the paper, and approved the final draft.
- Beginer Subhan contributed reagents/materials/analyses tools, conceived and designed the experiments, conceived the research idea, authored or reviewed drafts of the paper, and approved the final draft
- Hawis Madduppa contributed reagents/materials/analyses tools, conceived and designed the experiments, conceived the research idea, authored or reviewed drafts of the paper, and approved the final draft.

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 Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE) of IPB University, award no. AID-497-A-16-00004. The field research permit was issued by IPB University Rector (Surat Tugas no. 403/IT3/KP/2019). Permits for this research were issued by the Indonesian Ministry of Research and Technology to Erin Borbee (130/E5/E5.4/SIP/2019), Christopher Lane (461/SIP/FRP/E5/Dit.KI/XII/2017), and Austin T Humphries (455/SIP/FRP/E5/Dit.KI/XII/2017).

Data Availability

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

498 **REFERENCES**

499

500 Abrego D, Ulstrup KE, Willis BL, Van Oppen MJH. 2008. Species-specific interactions between
501 algal endosymbionts and coral hosts define their bleaching response to heat and light stress.
502 Proceedings of the Royal Society B: Biological Sciences 275:2273–2282. DOI:
503 10.1098/rspb.2008.0180.

504 Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM. 2009. A method for studying
505 protistan diversity using massively parallel sequencing of V9 hypervariable regions of
506 small-subunit ribosomal RNA Genes. *PLoS ONE* 4:1–9. DOI:
507 10.1371/journal.pone.0006372.

508 Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. 26:32–
509 46.

510 Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, LaJeunesse TC,
511 Voolstra CR. 2014. Assessing *Symbiodinium* diversity in scleractinian corals via next-
512 generation sequencing-based genotyping of the ITS2 rDNA region. *Molecular Ecology*
513 23:4418–4433. DOI: 10.1111/mec.12869.

514 Auliaherliaty L, Dewi KT, Priohandono YA. 2004. Foraminifera Di Teluk Sepi - Blongas,
515 Lombok Selatan, Nusa Tenggara Barat Dan Kaitannya Dengan Faktor Lingkungan. *Jurnal*
516 *Geologi Kelautan* 2:1–8. DOI: 10.32693/jgk.2.3.2004.115.

517 Baker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and
518 biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*
519 34:661–689. DOI: 10.1146/annurev.ecolsys.34.011802.132417.
520 Bálint M, Nowak C, Márton O, Pauls SU, Wittwer C, Aramayo JL, Schulze A, Chambert T,
521 Cocchiararo B, Jansen M. 2018. Accuracy, limitations and cost efficiency of eDNA-based
522 community survey in tropical frogs. *Molecular Ecology Resources* 18:1415–1426. DOI:
523 10.1111/1755-0998.12934.
524 Barneah O, Brickner I, Hooge M, Weis VM, LaJeunesse TC, Benayahu Y. 2007. Three party
525 symbiosis: Acoelomorph worms, corals and unicellular algal symbionts in Eilat (Red Sea).
526 *Marine Biology* 151:1215–1223. DOI: 10.1007/s00227-006-0563-2.
527 Barnes MA, Turner CR. 2016. The ecology of environmental DNA and implications for
528 conservation genetics. *Conservation Genetics* 17:1–17. DOI: 10.1007/s10592-015-0775-4.
529 Baskett ML, Gaines SD, Nisbet RM. 2009. Symbiont diversity may help coral reefs survive
530 moderate climate change. *Ecological Applications* 19:3–17. DOI: 10.1890/08-0139.1.
531 Bay LK, Doyle J, Logan M, Berkelmans R. 2016. Recovery from bleaching is mediated by
532 threshold densities of background thermo-tolerant symbiont types in a reef-building coral.
533 *Royal Society Open Science* 3:1603221–60322. DOI: 10.1098/rsos.160322.
534 Berkelmans R, Van Oppen MJH. 2006. The role of zooxanthellae in the thermal tolerance of
535 corals: A “nugget of hope” for coral reefs in an era of climate change. *Proceedings of the*

536 *Royal Society B: Biological Sciences* 273:2305–2312. DOI: 10.1098/rspb.2006.3567.

537 Bo M, Baker AC, Gaino E, Wirshing HH, Scoccia F, Bavestrello G. 2011. First description of

538 algal mutualistic endosymbiosis in a black coral (Anthozoa: Antipatharia). *Marine Ecology*

539 *Progress Series* 435:1–11. DOI: 10.3354/meps09228.

540 Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory

541 Caporaso J. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences

542 with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:1–17. DOI: 10.1186/s40168-

543 018-0470-z.

544 Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm

545 EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ,

546 Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener

547 C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M,

548 Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J,

549 Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK,

550 Jiang L, Kaehler BD, Kang K Bin, Keefe CR, Keim P, Kelley ST, Knights D, Koester I,

551 Kosciolek T, Kreps J, Langille MGI, Lee J, Ley RE, Liu Y-X, Loftfield E, Lozupone C,

552 Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik A V, Metcalf JL,

553 Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB,

554 Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson

555 MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford
556 AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der
557 Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y,
558 Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang
559 Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible
560 microbiome data science using QIIME 2. *Nature Biotechnology* 37:852–857. DOI:
561 10.1038/s41587-019-0209-9.

562 Bongaerts P, Carmichael M, Hay KB, Tonk L, Frade PR, Hoegh-Guldberg O. 2015. Prevalent
563 endosymbiont zonation shapes the depth distributions of scleractinian coral species. *Royal*
564 *Society Open Science* 2:140297–140297. DOI: 10.1098/rsos.140297.

565 Bourne DG, Morrow KM, Webster NS. 2016. Insights into the Coral Microbiome: underpinning
566 the ehalth and resilience of reef ecosystems. *Annual Review of Microbiology* 70:317–340.
567 DOI: 10.1146/annurev-micro-102215-095440.

568 Brian JI, Davy SK, Wilkinson SP. 2019. Elevated Symbiodiniaceae richness at Atauro Island
569 (Timor-Leste): a highly biodiverse reef system. *Coral Reefs* 38:123–136. DOI:
570 10.1007/s00338-018-01762-9.

571 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2:
572 High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–
573 583. DOI: 10.1038/nmeth.3869.

574 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña
575 AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE,
576 Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh
577 PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows
578 analysis of high-throughput community sequencing data. *Nature Methods* 7:335–336. DOI:
579 10.1038/nmeth.f.303.
580 Carlos AA, Baillie BK, Kawachi M, Maruyama T. 1999. Phylogenetic position of *Symbiodinium*
581 (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a
582 soft coral (Anthozoa), and a free-living strain. *Journal of Phycology* 35:1054–1062. DOI:
583 10.1046/j.1529-8817.1999.3551054.x.
584 Castro-Sanguino C, Sánchez JA. 2012. Dispersal of *Symbiodinium* by the stoplight parrotfish
585 *Sparisoma viride*. *Biology Letters* 8:282–286. DOI: 10.1098/rsbl.2011.0836.
586 Claar DC, Starko S, Tietjen KL, Epstein HE, Cunning R, Cobb KM, Baker AC, Gates RD, Baum
587 JK. 2020. Dynamic symbioses reveal pathways to coral survival through prolonged
588 heatwaves. *Nature Communications* 11:1–10. DOI: 10.1038/s41467-020-19169-y.
589 Coffroth MA, Lewis CF, Santos SR, Weaver JL. 2006. Environmental populations of symbiotic
590 dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians.
591 *Current Biology* 16:985–987. DOI: 10.1016/j.cub.2006.10.049.
592 Cunning R, Silverstein RN, Baker AC. 2015. Investigating the causes and consequences of

symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proceedings of the Royal Society B: Biological Sciences 282. DOI: 10.1098/rspb.2014.1725.

Da-Anoy JP, Cabaitan PC, Conaco C. 2019. Species variability in the response to elevated temperature of select corals in north-western Philippines. *Journal of the Marine Biological Association of the United Kingdom* 99:1273–1279. DOI: 10.1017/S0025315419000158.

Davy SK, Allemand D, Weis VM. 2012. Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews* 76:229 LP – 261. DOI: 10.1128/MMBR.05014-11.

DeBoer TS, Baker AC, Erdmann M V, Ambariyanto, Jones PR, Barber PH. 2012. Patterns of *Symbiodinium* distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. *Marine Ecology Progress Series* 444:117–132. DOI: 10.3354/meps09413.

Decelle J, Carradec Q, Pochon X, Henry N, Romac S, Mahé F, Dunthorn M, Kourlaiev A, Voolstra CR, Wincker P, de Vargas C. 2018. Worldwide occurrence and activity of the reef-building coral symbiont *Symbiodinium* in the open ocean. *Current Biology* 28:3625-3633.e3. DOI: 10.1016/j.cub.2018.09.024.

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L. 2017. Environmental DNA

612 metabarcoding: transforming how we survey animal and plant communities. *Molecular*
613 *Ecology* 26:5872–5895. DOI: 10.1111/mec.14350.
614 Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011.
615 Persistence of environmental DNA in freshwater ecosystems. *PLoS ONE* 6:8–11. DOI:
616 10.1371/journal.pone.0023398.
617 Dewi KT, Arifin L, Yuningsih A, Permanawati Y. 2012. Meiofauna (Foraminifera) in sediments
618 and its relation to white sandy beach of Senggigi and water condition off West Lombok.
619 *Jurnal Ilmu dan Teknologi Kelautan Tropis* 4:47–54. DOI: 10.29244/jitkt.v4i1.7805.
620 Fabricius KE, Mieog JC, Colin PL, Idip D, Van Oppen MJH. 2004. Identity and diversity of
621 coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching,
622 temperature and shading histories. *Molecular Ecology* 13:2445–2458. DOI: 10.1111/j.1365-
623 294X.2004.02230.x.
624 Freudenthal HD. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a
625 zooxanthella : taxonomy, life cycle, and morphology. *Protozoology* 9:45–52.
626 Fujise L, Suggett DJ, Stat M, Kahlke T, Bunce M, Gardner SG, Goyen S, Woodcock S, Ralph
627 PJ, Seymour JR, Siboni N, Nitschke MR. 2021. Unlocking the phylogenetic diversity,
628 primary habitats, and abundances of free-living Symbiodiniaceae on a coral reef. *Molecular*
629 *Ecology* 30:343–360. DOI: 10.1111/mec.15719.
630 Fujise L, Yamashita H, Suzuki G, Sasaki K, Liao LM, Koike K. 2014. Moderate thermal stress

631 causes active and immediate expulsion of photosynthetically damaged zooxanthellae
632 (*Symbiodinium*) from corals. *PLoS ONE* 9:1–18. DOI: 10.1371/journal.pone.0114321.
633 Gelis ERE, Kamal MM, Subhan B, Bachtiar I, Sani LMI, Madduppa H. 2021. Environmental
634 biomonitoring of reef fish community structure with eDNA metabarcoding in the Coral
635 Triangle. *Environmental Biology of Fishes* 104:887–903. DOI: 10.1007/s10641-021-01118-
636 3.
637 Giyanto, Abrar M, Hadi TA, Budiyanto A, Muhammad Hafizt, Salatalohy A, Iswari MY. 2017.
638 *Status terumbu karang di Indonesia 2017*. Jakarta: P2O-LIPI.
639 Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee
640 A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS,
641 Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P. 2016. Critical
642 considerations for the application of environmental DNA methods to detect aquatic species.
643 *Methods in Ecology and Evolution* 7:1299–1307. DOI: 10.1111/2041-210X.12595.
644 Grupstra CGB, Rabbitt KM, Howe-Kerr LI, Correa AMS. 2021. Fish predation on corals
645 promotes the dispersal of coral symbionts. *bioRxiv*. DOI: 10.1101/2020.08.10.243857.
646 Hirose M, Reimer JD, Hidaka M, Suda S. 2008. Phylogenetic analyses of potentially free-living
647 *Symbiodinium* spp. isolated from coral reef sand in Okinawa, Japan. *Marine Biology*
648 155:105–112. DOI: 10.1007/s00227-008-1011-2.
649 Hoadley KD, Lewis AM, Wham DC, Pettay DT, Grasso C, Smith R, Kemp DW, LaJeunesse TC,

650 Warner ME. 2019. Host–symbiont combinations dictate the photo-physiological response of
651 reef-building corals to thermal stress. Scientific Reports 9:1–16. DOI: 10.1038/s41598-019-
652 46412-4.
653 Howells EJ, Abrego D, Liew YJ, Burt JA, Meyer E, Aranda M. 2021. Enhancing the heat
654 tolerance of reef-building corals to future warming. *Science Advances* 7. DOI:
655 10.1126/sciadv.abg6070.
656 Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J. 2015. *Symbiodinium*
657 *thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's
658 hottest sea, the Persian/Arabian Gulf. *Scientific Reports* 5:1–8. DOI: 10.1038/srep08562.
659 Jeong HJ, Lee SY, Kang NS, Yoo Y Du, Lim AS, Lee MJ, Kim HS, Yih W, Yamashita H,
660 Lajeunesse TC. 2014. Genetics and morphology characterize the dinoflagellate
661 *Symbiodinium voratum*, n. sp., (dinophyceae) as the sole representative of *Symbiodinium*
662 clade e. *Journal of Eukaryotic Microbiology* 61:75–94. DOI: 10.1111/jeu.12088.
663 Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017. ModelFinder:
664 fast model selection for accurate phylogenetic estimates. *Nature Methods* 14:587–589. DOI:
665 10.1038/nmeth.4285.
666 Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7:
667 improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780.
668 DOI: 10.1093/molbev/mst010.

669 Kimes NE, Johnson WR, Torralba M, Nelson KE, Weil E, Morris PJ. 2013. The *Montastraea*
670 *faveolata* microbiome: ecological and temporal influences on a Caribbean reef-building
671 coral in decline. *Environmental Microbiology* 15:2082–2094. DOI: 10.1111/1462-
672 2920.12130.
673 Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-
674 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on
675 the miseq illumina sequencing platform. *Applied and Environmental Microbiology*
676 79:5112–5120. DOI: 10.1128/AEM.01043-13.
677 Kruskal WH, Wallis WA. 1952. Journal of the American use of ranks in one-criterion variance
678 aalysis. *Journal of The American Statistical Association*:37–41. DOI:
679 10.1080/01621459.1952.10483441.
680 Kurihara H, Watanabe A, Tsugi A, Mimura I, Hongo C, Kawai T, Reimer JD, Kimoto K,
681 Gouezo M, Golbuu Y. 2021. Potential local adaptation of corals at acidified and warmed
682 Nikko Bay, Palau. *Scientific Reports* 11:1–11. DOI: 10.1038/s41598-021-90614-8.
683 LaJeunesse TC. 2005. “Species” radiations of symbiotic dinoflagellates in the Atlantic and Indo-
684 Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution* 22:570–
685 581. DOI: 10.1093/molbev/msi042.
686 LaJeunesse TC, Bhagooli R, Hidaka M, DeVantier L, Done T, Schmidt G, Fitt W, Hoegh-
687 Guldberg O. 2004. Closely related *Symbiodinium* spp. differ in relative dominance in coral

reef host communities across environmental, latitudinal and biogeographic gradients. *Marine Ecology-Progress Series*- 284:147–161.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR. 2018. Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology* 28:2570-2580.e6. DOI: 10.1016/j.cub.2018.07.008.

LaJeunesse TC, Parkinson JE, Trench RK. 2012. *Symbiodinium*. Version 04 July 2012. *Available at http://tolweb.org/Symbiodinium/126705/2012.07.04 in The Tree of Life Web Project, http://tolweb.org/*

LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O, Fitt WK. 2010. Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. *Journal of Biogeography* 37:785–800. DOI: 10.1111/j.1365-2699.2010.02273.x.

LaJeunesse TC, Smith R, Walther M, Pinzón J, Pettay DT, McGinley M, Aschaffenburg M, Medina-Rosas P, Cupul-Magaña AL, Pérez AL, Reyes-Bonilla H, Warner ME. 2010. Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. Proceedings of the Royal Society B: Biological Sciences 277:2925–2934. DOI: 10.1098/rspb.2010.0385.

LaJeunesse TC, Wiedenmann J, Casado-Amezúa P, D'Ambra I, Turnham KE, Nitschke MR,

707 Oakley CA, Goffredo S, Spano CA, Cubillos VM, Davy SK, Suggett DJ. 2021. Revival of
708 *Philozoon Geddes* for host-specialized dinoflagellates, 'zooxanthellae', in animals from
709 coastal temperate zones of northern and southern hemispheres. *European Journal of*
710 *Phycology*:1–15. DOI: 10.1080/09670262.2021.1914863.
711 Lesser MP, Stat M, Gates RD. 2013. The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of
712 corals are parasites and mutualists. *Coral Reefs* 32:603–611. DOI: 10.1007/s00338-013-
713 1051-z.
714 Littman RA, van Oppen MJH, Willis BL. 2008. Methods for sampling free-living *Symbiodinium*
715 (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef).
716 *Journal of Experimental Marine Biology and Ecology* 364:48–53. DOI:
717 10.1016/j.jembe.2008.06.034.
718 Loh WKW, Cowlishaw M, Wilson NG. 2006. Diversity of *Symbiodinium* dinoflagellate
719 symbionts from the Indo-Pacific sea slug *Pteraeolidia ianthina* (Gastropoda: Mollusca).
720 *Marine Ecology Progress Series* 320:177–184. DOI: 10.3354/meps320177.
721 Madduppa H, Cahyani NKD, Anggoro AW, Subhan B, Jefri E, Sani LMI, Arafat D, Akbar N,
722 Bengen DG. 2021. eDNA metabarcoding illuminates species diversity and composition of
723 three phyla (chordata, mollusca and echinodermata) across Indonesian coral reefs.
724 *Biodiversity and Conservation* 30:3087–3114. DOI: 10.1007/s10531-021-02237-0.
725 Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.

726 *EMBnet* 17. DOI: 10.14806/ej.17.1.200.

727 Minh B, Trifinopoulos J, Schrempf D, Schmidt HA. 2021. IQ-TREE version 2.1.2: tutorials and
728 manual phylogenomic software by maximum likelihood.

729 Montresor M, Lovejoy C, Orsini L, Procaccini G, Roy S. 2003. Bipolar distribution of the cyst-
730 forming dinoflagellate *Polarella glacialis*. *Polar Biology* 26:186–194. DOI:
731 10.1007/s00300-002-0473-9.

732 Natsir SM. 2009. First record of agglutinated foraminifera from Lombok. *Journal of Coastal*
733 *Development* 13:46–53.

734 Natsir SM. 2010. Distribusi foraminifera bentik resen di Perairan Lombok (suatu tinjauan di
735 daerah Gili Air, Gili Meno dan Gili Trawangan). *Biosfera* 27:95–102. DOI:
736 10.20884/1.mib.2010.27.2.198.

737 Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective
738 stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology*
739 *and Evolution* 32:268–274. DOI: 10.1093/molbev/msu300.

740 Nitschke MR, Craveiro SC, Brandão C, Fidalgo C, Serôdio J, Calado AJ, Frommlet JC. 2020.
741 Description of *Freudenthalidium* gen. nov. and *Halluxium* gen. nov. to formally recognize
742 Clades Fr3 and H as genera in the family Symbiodiniaceae (Dinophyceae). *Journal of*
743 *Phycology* 56:923–940. DOI: 10.1111/jpy.12999.

744 Pandeirada MS, Craveiro SC, Calado AJ. 2013. Freshwater dinoflagellates in Portugal (W

745 Iberia): a critical checklist and new observations. *Nova Hedwigia* 97:321–348. DOI:
746 10.1127/0029-5035/2013/0119.
747 Pochon X, Pawlowski J, Zaninetti L, Rowan R. 2001. High genetic diversity and relative
748 specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid
749 foraminiferans. Marine Biology 139:1069–1078. DOI: 10.1007/s002270100674.
750 Pochon X, Garcia-Cuetos L, Baker AC, Castella E, Pawlowski J. 2007. One-year survey of a
751 single Micronesian reef reveals extraordinarily rich diversity of *Symbiodinium* types in
752 soritid foraminifera. *Coral Reefs* 26:867–882. DOI: 10.1007/s00338-007-0279-x.
753 Pochon X, Gates RD. 2010. A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera
754 in Hawai'i. *Molecular Phylogenetics and Evolution* 56:492–497. DOI:
755 10.1016/j.ympev.2010.03.040.
756 Pochon X, LaJeunesse TC. 2021. *Miliolidium* n. gen, a New Symbiodiniacean Genus Whose
757 Members Associate with Soritid Foraminifera or Are Free-Living. *Journal of Eukaryotic*
758 *Microbiology* 68:0–1. DOI: 10.1111/jeu.12856.
759 Pochon X, LaJeunesse TC, Pawlowski J. 2004. Biogeographic partitioning and host
760 specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta).
761 *Marine Biology* 146:17–27. DOI: 10.1007/s00227-004-1427-2.
762 Pochon X, Pawlowski J. 2006. Review article Evolution of the soritids- *Symbiodinium*
763 symbiosis. *Symbiosis*:77–88.

764 Pochon X, Putnam HM, Gates RD. 2014. Multi-gene analysis of *Symbiodinium* dinoflagellates:
765 A perspective on rarity, symbiosis, and evolution. *PeerJ* 2014:1–25. DOI:
766 10.7717/peerj.394.
767 Purnomo PW. 2014. Translocation study of some zooxanthellae clade to the survival and growth
768 of *Goniastrea aspera* after bleaching. *International Journal of Marine and Aquatic*
769 *Resource Conservation and Co-existence* 1:50–56. DOI: doi.org/10.14710/ijfst.7.1.39-45.
770 Rajan V. 2012. A method of alignment masking for refining the phylogenetic signal of multiple
771 sequence alignments. *Molecular Biology and Evolution* 30:689–712. DOI:
772 10.1093/molbev/mss264.
773 Ramsby BD, Hill MS, Thornhill DJ, Steenhuizen SF, Achlatis M, Lewis AM, LaJeunesse TC.
774 2017. Sibling species of mutualistic *Symbiodinium* clade G from bioeroding sponges in the
775 western Pacific and western Atlantic oceans. *Journal of Phycology* 53:951–960. DOI:
776 10.1111/jpy.12576.
777 Ravelo SF, Conaco C. 2018. Comparison of the response of in hospite and ex hospite
778 *Symbiodinium* to elevated temperature. *Marine and Freshwater Behaviour and Physiology*
779 51:93–108. DOI: 10.1080/10236244.2018.1503935.
780 Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. The detection of
781 aquatic animal species using environmental DNA - a review of eDNA as a survey tool in
782 ecology. *Journal of Applied Ecology* 51:1450–1459. DOI: 10.1111/1365-2664.12306.

783 Reimer JD, Todd P. 2009. Preliminary molecular examination of zooxanthellate zoanthid
784 (Hexacorallia, Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in
785 Singapore. *Raffles Bulletin of Zoology* 22:103–120.
786 Rouzé H, Lecellier GJ, Saulnier D, Planes S, Gueguen Y, Wirshing HH, Berteaux-Lecellier V.
787 2017. An updated assessment of *Symbiodinium* spp. that associate with common
788 scleractinian corals from moorea (French Polynesia) reveals high diversity among
789 background symbionts and a novel finding of clade B. *PeerJ* 5:e2856. DOI:
790 10.7717/peerj.2856.
791 Rowan R, Powers DA. 1991. A molecular genetic classification of zooxanthellae and the
792 evolution of animal-algal symbioses. *Science* 251:1348–1351. DOI:
793 10.1126/science.251.4999.1348.
794 Sakata MK, Yamamoto S, Gotoh RO, Miya M, Yamanaka H, Minamoto T. 2020. Sedimentary
795 eDNA provides different information on timescale and fish species composition compared
796 with aqueous eDNA. *Environmental DNA* 2:505–518. DOI: 10.1002/edn3.75.
797 Schönberg CHL, Loh WKW. 2005. Molecular identity of the unique symbiotic dinoflagellates
798 found in the bioeroding demosponge *Cliona orientalis*. *Marine Ecology Progress Series*
799 299:157–166. DOI: 10.3354/meps299157.
800 Shinzato C, Zayasu Y, Kanda M, Kawamitsu M, Satoh N, Yamashita H, Suzuki G. 2018. Using
801 seawater to document coral-zoothanthella diversity: a new approach to coral reef

802 monitoring using environmental DNA. *Frontiers in Marine Science* 5:1–12. DOI:
803 10.3389/fmars.2018.00028.
804 Smart AS, Weeks AR, van Rooyen AR, Moore A, McCarthy MA, Tingley R. 2016. Assessing
805 the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution*
806 7:1291–1298. DOI: 10.1111/2041-210X.12598.
807 Stat M, Gates RD. 2011. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a
808 selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology*
809 2011:730715. DOI: 10.1155/2011/730715.
810 Stat M, Morris E, Gates RD. 2008. Functional diversity in coral-dinoflagellate symbiosis.
811 *Proceedings of the National Academy of Sciences of the United States of America*
812 105:9256–9261. DOI: 10.1073/pnas.0801328105.
813 Stoeck T, Bass D, Nebel M, Christen R, Meredith D. 2010. Multiple marker parallel tag
814 environmental DNA sequencing reveals a highly complex eukaryotic community in marine
815 anoxic water. *Molecular Ecology* 19:21-31. DOI: 10.1111/j.1365-294X.2009.04480.x.
816 Strickler KM, Fremier AK, Goldberg CS. 2014. Quantifying effects of UV-B, temperature, and
817 pH on eDNA degradation in aquatic microcosms. *Biological Conservation* 183:85–92. DOI:
818 10.1016/j.biocon.2014.11.038.
819 Suggett DJ, Warner ME, Leggat W. 2017. Symbiotic Dinoflagellate Functional Diversity
820 Mediates Coral Survival under Ecological Crisis. *Trends in Ecology and Evolution* 32:735–

745. DOI: 10.1016/j.tree.2017.07.013.

Suzuki G, Yamashita H, Kai S, Hayashibara T, Suzuki K, Iehisa Y, Okada W, Ando W, Komori T. 2013. Early uptake of specific symbionts enhances the post-settlement survival of *Acropora* corals. *Marine Ecology Progress Series* 494:149–158. DOI: 10.3354/meps10548.

Swain TD, Chandler J, Backman V, Marcelino L. 2017. Consensus thermotolerance ranking for 110 *Symbiodinium* phylotypes: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential. *Functional Ecology* 31:172–183. DOI: 10.1111/1365-2435.12694.

Taguba CA, Sotto FB, Geraldino PJL. 2016. Identification of *Symbiodinium* clade of the blue coral *Heliopora Coerulea* (Pallas, 1766) (Helioporacea: Helioporidae) from surrounding waters in Central Visayas, Philippines. In: *Developing life–supporting marine ecosystems along the Asia–Pacific coasts – a synthesis of physical and biological data for the science–based management and socio– ecological policy making*. 94–98.

Takabayashi M, Adams LM, Pochon X, Gates RD. 2012. Genetic diversity of free-living *Symbiodinium* in surface water and sediment of Hawai'i and Florida. *Coral Reefs* 31:157–167. DOI: 10.1007/s00338-011-0832-5.

Tan YTR, Wainwright BJ, Afiq-Rosli L, Ip YCA, Lee JN, Nguyen NTH, Pointing SB, Huang D. 2020. Endosymbiont diversity and community structure in *Porites lutea* from Southeast Asia are driven by a suite of environmental variables. *Symbiosis* 80:269–277. DOI:

10.1007/s13199-020-00671-2.

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* 7. DOI: 10.1371/journal.pone.0041732.

Tong F, Zhang L, Chen PM, Chen WJ. 2018. Molecular taxonomy and diversity of *Symbiodinium* spp. based on 28S rDNA sequences within 15 coral species in Daao Bay, Shenzhen. In: *IOP Conference Series: Materials Science and Engineering*. DOI: 10.1088/1757-899X/392/4/042033.

Torti A, Lever MA, Jørgensen BB. 2015. Origin, dynamics, and implications of extracellular DNA pools in marine sediments. *Marine Genomics* 24:185–196. DOI: 10.1016/j.margen.2015.08.007.

Turner CR, Barnes MA, Xu CCY, Jones SE, Jerde CL, Lodge DM. 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5:676–684. DOI: 10.1111/2041-210X.12206.

Turner CR, Uy KL, Everhart RC. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation* 183:93–102. DOI: 10.1016/j.biocon.2014.11.017.

Venera-Ponton DE, Diaz-Pulido G, Rodriguez-Lanetty M, Hoegh-Guldberg O. 2010. Presence of *Symbiodinium* spp. in macroalgal microhabitats from the southern Great Barrier Reef.

Coral Reefs 29:1049–1060. DOI: 10.1007/s00338-010-0666-6.

Veron JEN, DeVantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N. 2009. Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies* 11:91–100. DOI: 10.3755/galaxea.11.91.

Yamashita H, Koike K. 2013. Genetic identity of free-living *Symbiodinium* obtained over a broad latitudinal range in the Japanese coast. *Phycological Research* 61:68–80. DOI: 10.1111/pre.12004.

Yorifuji M, Yamashita H, Suzuki G, Kawasaki T, Tsukamoto T, Okada W, Tamura K, Nakamura R, Inoue M, Yamazaki M, Harii S. 2021. Unique environmental Symbiodiniaceae diversity at an isolated island in the northwestern Pacific. Molecular Phylogenetics and Evolution 161:107158. DOI: 10.1016/j.ympev.2021.107158.

Figure 1

Map of the research sites around Lombok Island, Indonesia.

(A) West Lombok, (B) North Lombok, and (C) East Lombok.

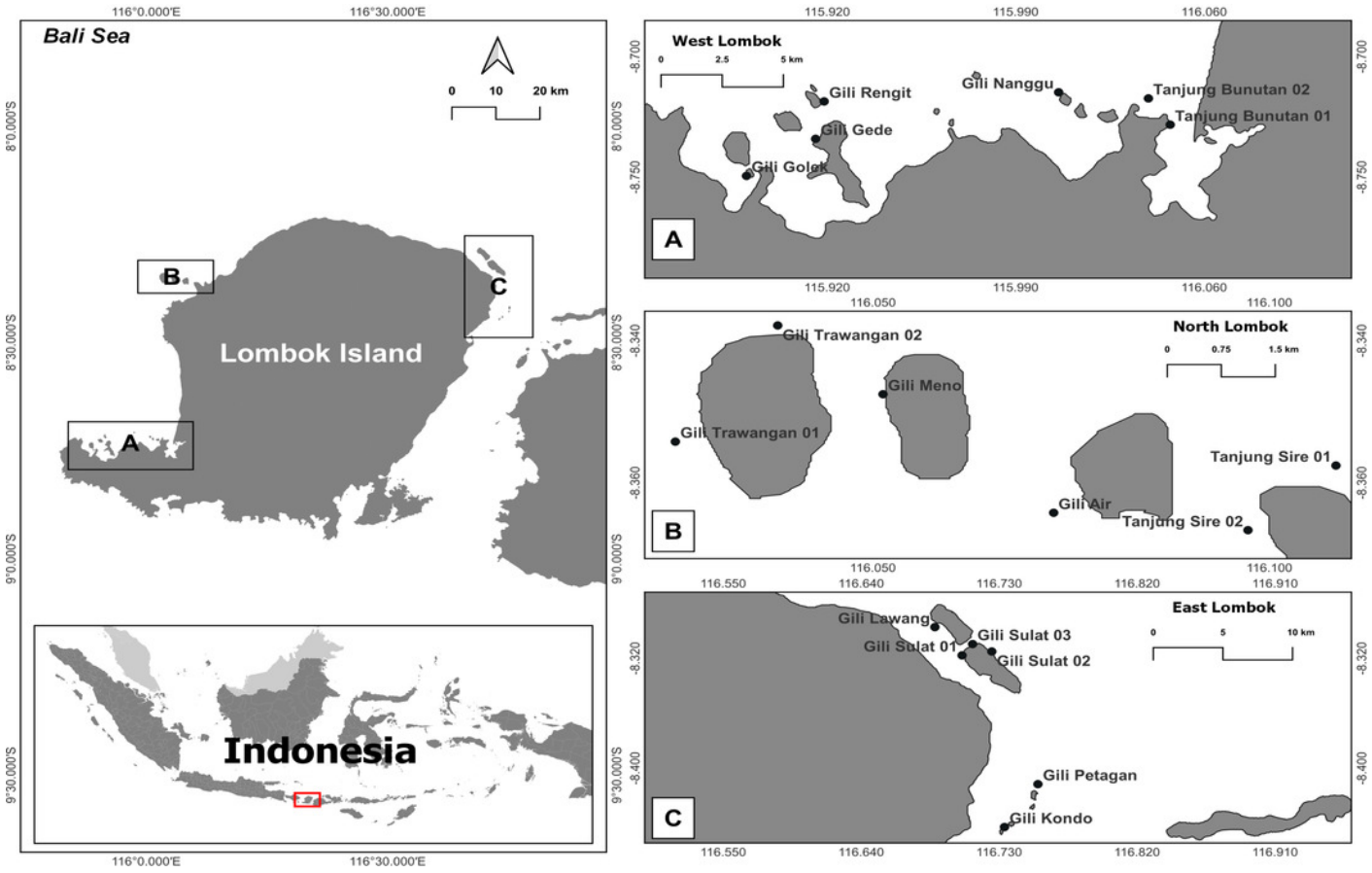

Figure 2

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/).

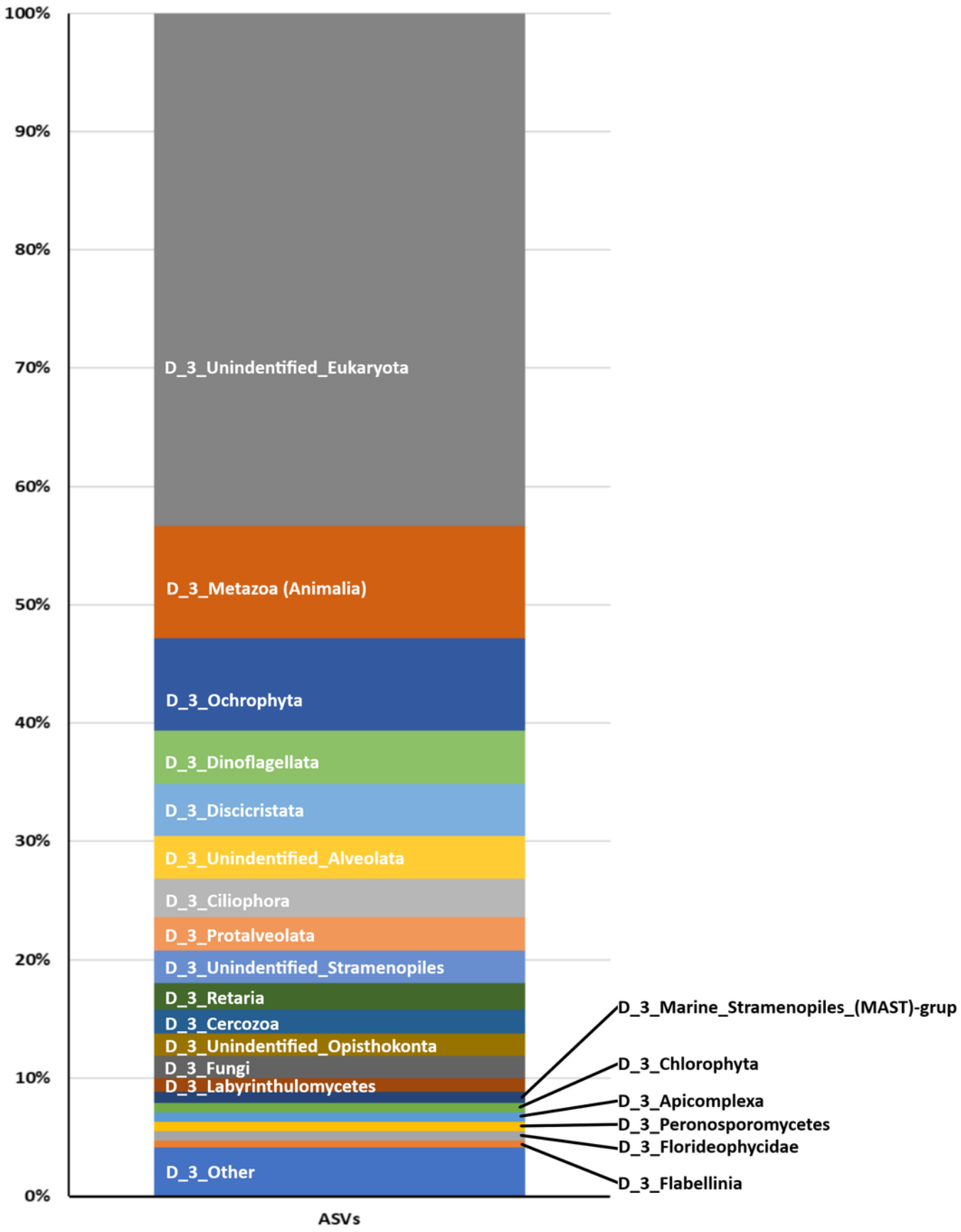
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
ASVs
D_3_Unindentified_Eukaryota
D_3_Metazoa (Animalia)
D_3_Ochrophyta
D_3_Dinoflagellata
D_3_Discicristata
D_3_Unindentified_Alveolata
D_3_Ciliophora
D_3_Protalveolata
D_3_Unindentified_Stramenopiles
D_3_Retaria
D_3_Cercozoa
D_3_Unindentified_Opisthokonta
D_3_Fungi
D_3_Labyrinthulomycetes
D_3_Other
D_3_Marine_Stramenopiles_(MAST)-grup
D_3_Chlorophyta
D_3_Apicomplexa
D_3_Peronosporomycetes
D_3_Florideophycidae
D_3_Flabellinia

Figure 3

Maximum likelihood phylogenetic tree based on V9-18S rRNA gene for Order Suessiales (A) and Family Symbiodiniaceae (B).

(A) Phylogeny of families in Order Suessiales. ASVs from this study (OTU.sym1–OTU.sym22) are shown in bold black font, and the branch support values 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) Phylogeny of genera in Family Symbiodiniaceae. ASVs are shown in bold black, 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/).

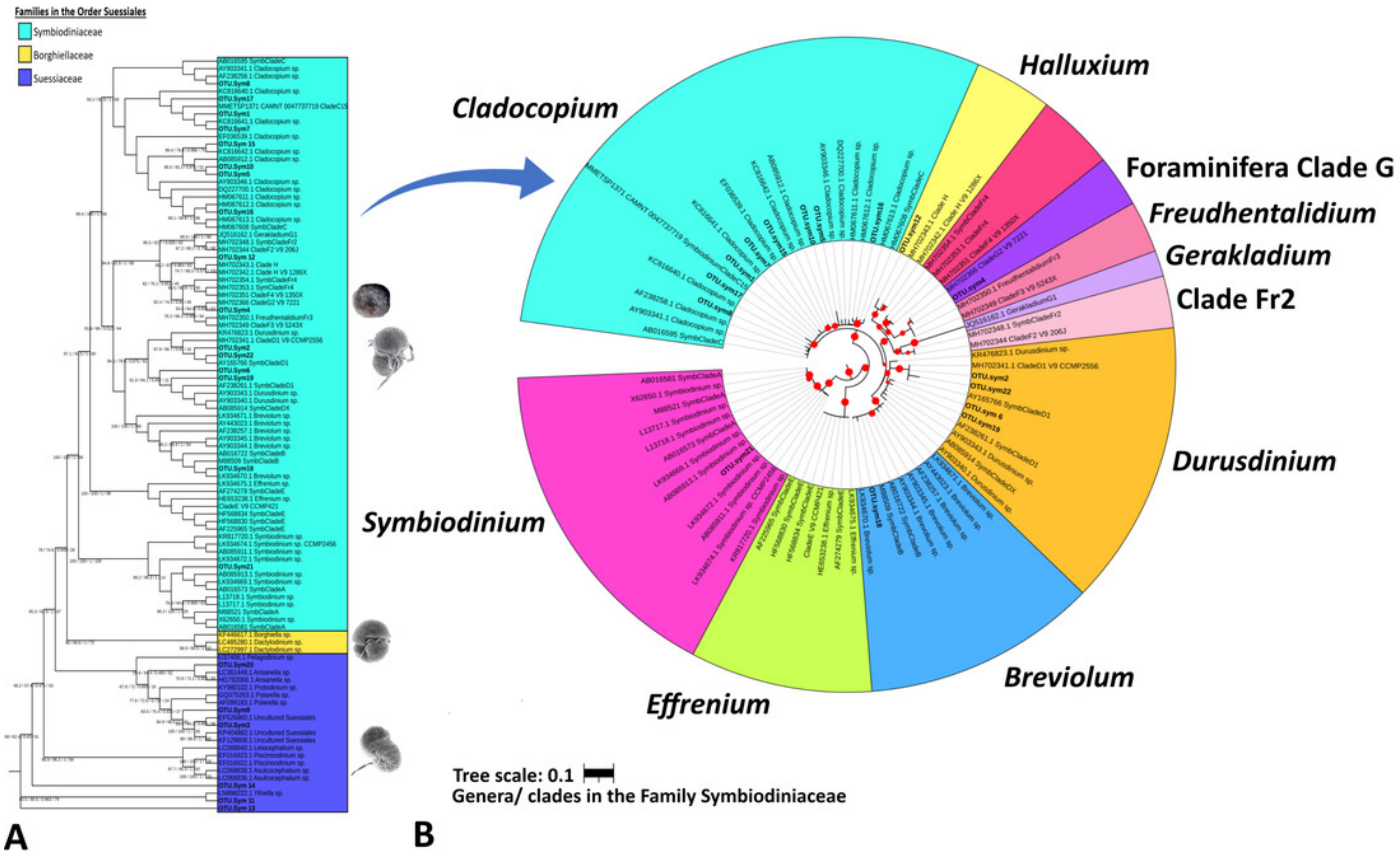
Families in the Order Suessiales
Symbiodiniaceae
Borghiellaceae
Suessiaceae
A
Cladocopium
Halluxium
Foraminifera Clade G
Freudhentalidium
Gerakladium
Clade Fr2
Durusdinium
Breviolum
Effrenium
Symbiodinium
Tree scale: 0.1
Genera/ clades in the Family Symbiodiniaceae
B

Figure 4

Venn diagram of Symbiodiniaceae subclades around Lombok by: (A) coastal area and (B) method (sample type–filter pore size combination).

Sample labels: sea = seawater sample; sed = sediment sample; _0.4 and _12 indicate the pore size of the filter (in µm).

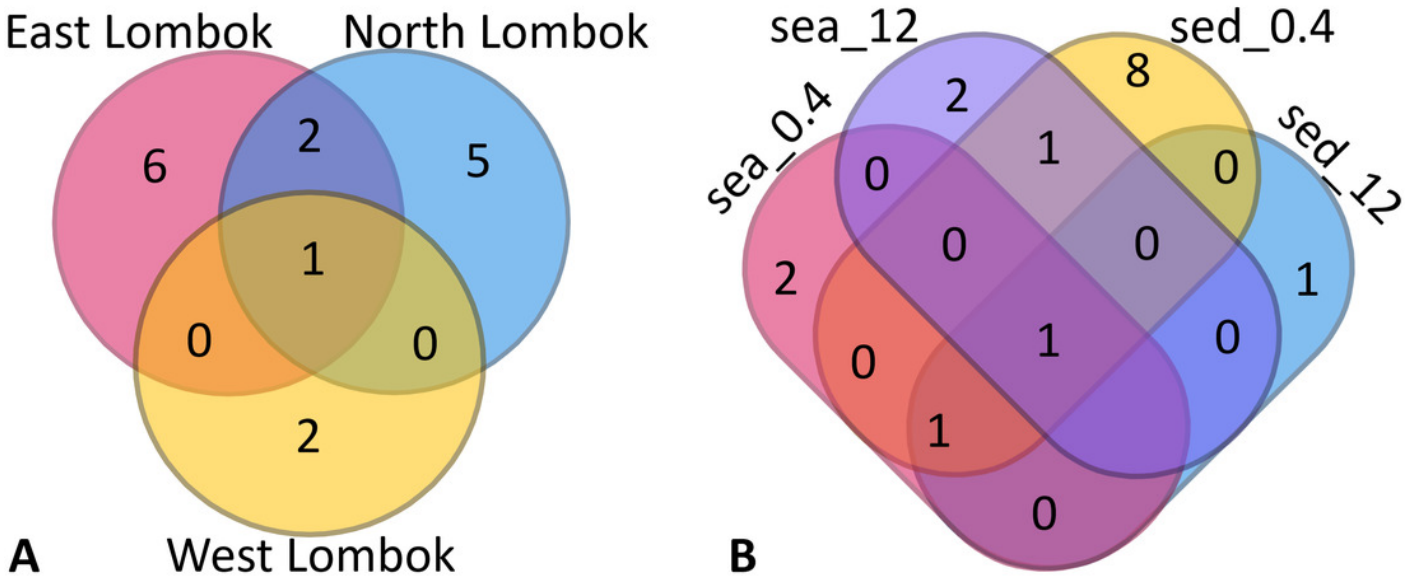

Figure 5

Composition of the relative abundance of Symbiodiniaceae communities across different sites, sample types, and fractions.

Relative abundance based on the 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.

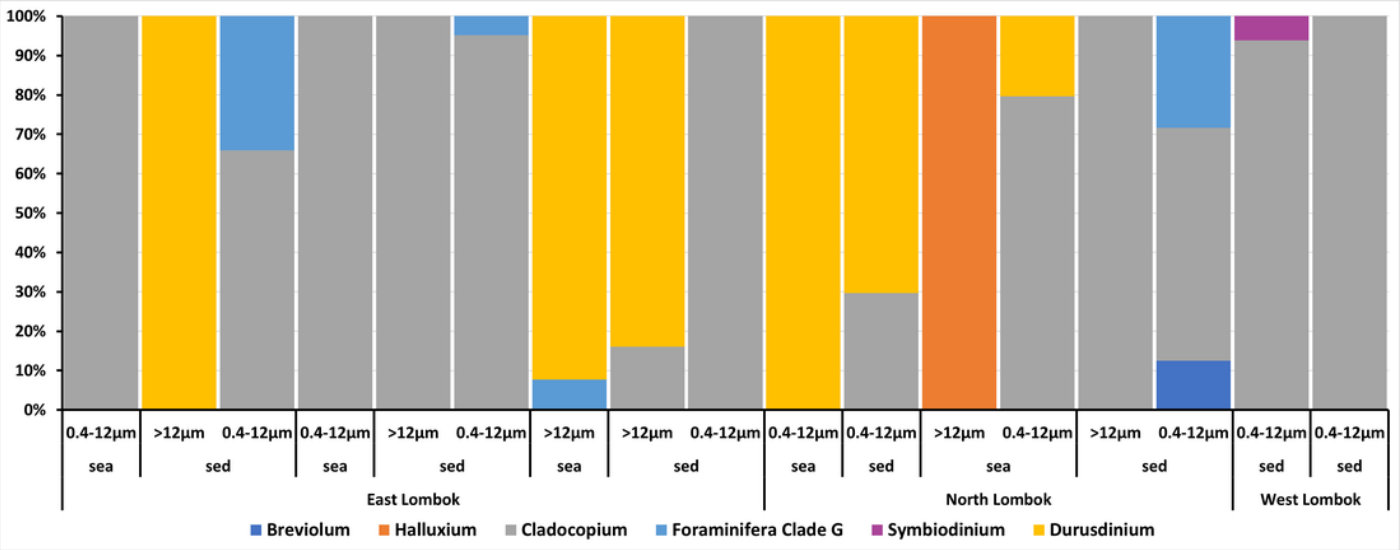

Figure 6

Total diversity of Symbiodiniaceae in coral reefs waters around Lombok Island: (A) alpha diversity and (B) beta diversity.

Alpha diversity is indicated by Shannon index and beta diversity is represented by Bray-Curtis (BC) dissimilarity. Boxplots display the median as the midline, and the upper and lower quartiles as the top and bottom lines of the boxes, respectively. Crossing symbols indicate the mean, and circles denote the outliers.

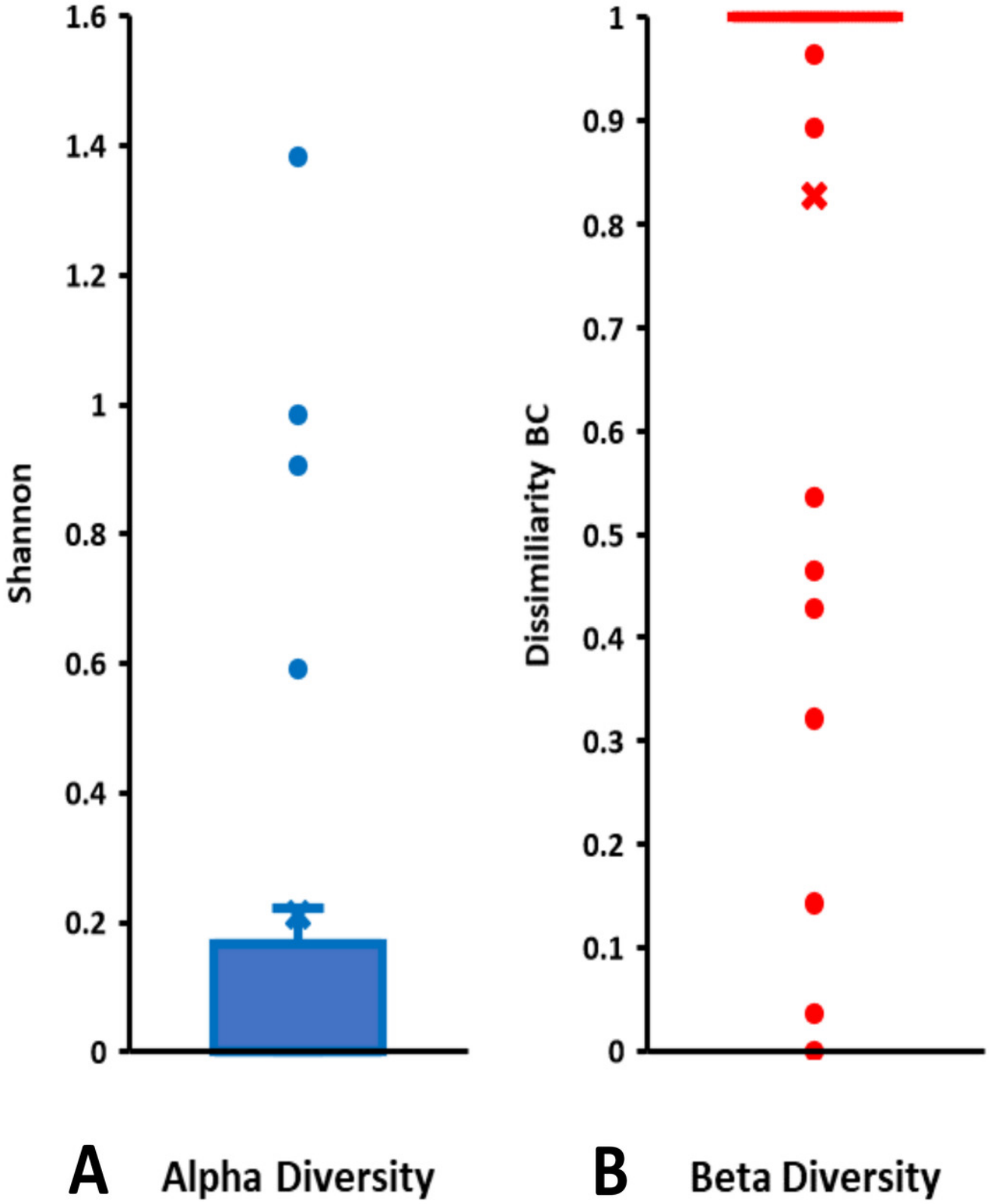
Shannon
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A Alpha Diversity
Dissimiliarity BC
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
B Beta Diversity

Table 1(on next page)

Coordinates of sampling stations at each coastal area around Lombok Island, Indonesia.

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(on next page)

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.
	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.

1

Table 3(on next page)

Summary of Symbiodiniaceae classifications.

ASVs were classified using a probabilistic Bayesian method referring to SILVA database at similarities of 97% and 99%, NCBI database BLAST routine, and phylogenetic reconstruction.

Notes: 1 In SILVA 97%, OTU.sym13 was classified as *Symbiodinium*, but in SILVA 99%, OTU.sym13 and OTU.sym11 were classified as *Polarella*. Therefore, the further analysis considered *Polarella* as possibly belonging to the Symbiodiniaceae. 2 According to Decelle et al. (2018). 3 Non BLAST result. 4 Nearest subclade branch (see Fig. 3). * Reference database. ** Confidence level. *** Percentage identity.

Methods →	Fit-Classifier-Naïve Bayes		BLAST			Phylogenetics	
DB Ref.* → OTUs	SILVA (97&99%)	Conf. rates**	Accession no.	NCBI[4]	%Id.***	Genera/subclades	Scores: SH-alrt/lbt/abayes/ufboot
OTU.sym1	Symbiodinium	0 .999410455	KC816641.1	*Cladocopium* sp. clade C	100	*Cladocopium*/C.sym1	93.2/90.8/1/69
OTU.sym2	Symbiodinium	0.823380237	AY165766.1	*Symbiodinium* sp. ex *P. briareum*/D1[2]	100	*Durusdinium*/D1.sym2	84.5/79.8/0.875/62
OTU.sym3	Symbiodinium	0.890746449	EF526860.1	uncultured marine Eukaryote	99.24	Unclassified Suessiaceae/OTU.sym3	100/100/1/100
OTU.sym4	Symbiodinium	0.78273645	MH702366	CladeG2_V9_7221[3]		Formaninifera Clade G/. G2.sym4	54.8/64.4/0.838/63
OTU.sym5	Symbiodinium	0.768964536	AB085912.1	*Cladocopium* sp.[3]		*Cladocopium*/C.sym5	93.2/90.8/1/69
OTU.sym6	Symbiodinium	0.993577182	AF238261.1	*Symbiodinium* sp. clade E/D1[2]	99.24	*Durusdinium*/D1.sym6	84.5/79.8/0.875/62
OTU.sym7	Symbiodinium	0.995164623	KC816641.1	*Cladocopium* sp. clade C	99.24	*Cladocopium*/C.sym7	93.2/90.8/1/69
OTU.sym8	Symbiodinium	0.999188819	AF238258.1	*Symbiodinium* sp. type C	99.24	*Cladocopium*/C.sym8	93.2/90.8/1/69
OTU.sym9	Symbiodinium	0.82770918	KP404862.1	uncultured Eukaryote[3]		Unclassified Suessiaceae/OTU.sym9	94.9/96.6/1/91
OTU.sym10	Symbiodinium	0.925078226	AB085912.1	*Cladocopium* sp.[3]		*Cladocopium*/C.sym10	93.2/90.8/1/69
OTU.sym11	Symbiodinium[1]	0.998952834	LN898222.1	*Yihiella yeosuensis*	100	*Yihiella*/OTU.sym11	83.5/80.5/0.963/79
OTU.sym12	Symbiodinium	0.991677932	MH702343.1	*Symbiodinium* sp. clade H	97.71	*Halluxium*/H.sym12	89.2/87/0.964/83
OTU.sym13	Symbiodinium[1]	0.942758624	LN898222.1	*Yihiella yeosuensis*	99.24	*Yihiella*/OTU.sym13	83.5/80.5/0.963/79
OTU.sym14	Symbiodinium	0.890990315		Incertae Sedis		Unclassified Suessiaceae/OTU.sym14	68/62.4/0.43/91
OTU.sym15	Symbiodinium	0.999046044	KC816642.1	*Cladocopium* sp. clade C	99.24	*Cladocopium*/C.sym15	93.2/90.8/1/69
OTU.sym16	Symbiodinium	0.999527293	HM067612.1	*Symbiodinium* sp. 2-125/CladeC[2]	99.24	*Cladocopium*/C.sym16	93.2/90.8/1/69
OTU.sym17	Symbiodinium	0.987978975	MMETSP1371	Symbiodinium C15[3]		*Cladocopium*/C.sym17	93.2/90.8/1/69
OTU.sym18	Symbiodinium	0.997997906	LK934670.1	*Breviolum minutum*	99.24	*Breviolum*/B.sym18	100/100/1/99
OTU.sym19	Symbiodinium	0.994027696	AF238261.1	*Symbiodinium* sp. clade E/D1[2]	99.24	*Durusdinium*/D1.sym19	84.5/79.8/0.875/62
OTU.sym20	Symbiodinium	0.742593275	LC361448.1	*Ansanella natalensis*	98.47	*Ansanella*/OTU.sym20	26.8/54.4/0.465/52
OTU.sym21	Symbiodinium	0.978735399	AB085913.1	*Cladocopium* sp.[3]		*Symbiodinium*/A.sym21	100/100/1/100
OTU.sym22	Symbiodinium	0.977772371	AY165766.1	*Symbiodinium* sp. ex *P. briareum*/D1[2]	100	*Durusdinium*/D1.sym22	84.5/79.8/0.875/62

Notes:

1 In SILVA 97%, OTU.sym13 was classified as Symbiodinium, but in SILVA 99%, OTU.sym13 and OTU.sym11 were classified as *Polarella*. Therefore, the further analysis considered *Polarella* as possibly belonging to the Symbiodiniaceae.

2 According to Decelle et al. (2018).

3 Non BLAST result.

4 Nearest subclade branch (see Fig. 3).

* Reference database

** Confidence level

*** Percentage identity

Table 4(on next page)

List of Symbiodiniaceae proportion per subclade based on present/absent analysis by site–sample type–filter pore size combination.

Symbiodiniaceae types considered as “common” were ≥33.33% in the presence of all sample combinations, and unique were <33.33% in the presence of all sample combinations. Sample label: ESea0.4 indicate site–sample type–filter pore size combination of East Lombok_Sea Water_0.4–12 µm; ESea12: East Lombok_Sea Water_>12 µm; ESed0.4: East Lombok_Sediment_0.4–12 µm; ESed12: East Lombok_Sediment_>12 µm; NSea0.4: North Lombok_Sea Water_0.4–12 µm; NSea12: North Lombok_ Sea Water_>12 µm; NSed0.4: North Lombok_Sediment_0.4–12 µm; NSed12: North Lombok_Sediment_>12 µm; WSed0.4: West Lombok_Sediment_0.4–12 µm.

Intersection inter site-sample type-fraction	Proportion per subclade (%)	Total	Subclades	Type
ESea0.4/Esed0.4/Esed12/NSea0.4/NSed0.4/NSed12/WSed0.4	77.78	1	C.sym1	Common
ESea12/ESed12/NSea0.4/NSed0.4	44.44	1	D1.sym2	Common
ESea12/Esed0.4/NSed0.4	33.33	1	G2.sym4	Common
ESea0.4	11.11	1	C.sym16	Unique
ESea12	11.11	1	D1.sym6	Unique
ESed0.4	11.11	3	C.sym7	Unique
	11.11		C.sym17	Unique
	11.11		C.sym10	Unique
ESed12	11.11	1	C.sym8	Unique
NSea0.4	11.11	1	D1.sym19	Unique
NSea12	11.11	1	H.sym12	Unique
NSed0.4	11.11	3	D1.sym22	Unique
	11.11		C.sym15	Unique
	11.11		B.sym18	Unique
WSed0.4	11.11	2	A.sym21	Unique
	11.11		C.sym5	Unique

1

Table 5(on next page)

Comparison of Symbiodiniaceae studies in Indonesia and Southeast Asia region.

Geographic scope	Sample type(s)	Identification Method(s)	Genera	Reference(s)
Indonesia:				
Sulawesi	Sea slugs (*Pteraeolidia ianthina*)	SSU 18S rRNA, LSU 28S rRNA, Single Stranded Conformational Polymorphism (SSCP)	*Cladocopium*, *Durusdinium*	Loh et al. 2006
West Sumatra	Anthipatharian black corals (*Cirrhipathes* sp.)	ITS2 rRNA, LSU 28S rRNA, denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphisms (RFLPs)	*Gerakladium*	Bo et al. 2011
Papua & West Papua	Giant clams (*Tridacna* spp.)	ITS2 rRNA, DGGE	*Symbiodinium*, *Breviolum*, *Cladocopium*	DeBoer et al. 2012
Central Java	Scleractinian corals, sea anemones, *Tridacna* sp.	SSU 18S rRNA, RFLPs	*Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*	Purnomo 2014
West Nusa Tenggara	Seawater, sediment	V9-SSU 18S rRNA	*Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Gerakladium*, *Halluxium*	This Study
Southeast Asia:				
Palau	Sponges (porifera), giant clams (*Tridacna* spp.), other bivalves (cardiids), foraminifera (*Amphisorus hemprichii*)	SSU 18S rRNA, RFLPs	*Symbiodinium*, *Cladocopium*, *Durusdinium*	Carlos et al. 1999
	Scleractinian corals	ITS1 rRNA, SSCP	*Cladocopium*, *Durusdinium*	Fabricius et al. 2004
	Scleractinian corals (*Porites cylindrica*)	ITS2 rRNA, psbA^{ncr}	*Cladocopium*, *Durusdinium*	Kurihara et al. 2021
Singapore	Sea slugs (*Pteraeolidia ianthina*)	SSU 18S rRNA, LSU 28S rRNA, SSCP	*Cladocopium*, *Durusdinium*	Loh et al. 2006
	Zoantharians	mt 16S rRNA, mt COI, ITS rRNA	*Cladocopium*, *Durusdinium*	Reimer and Todd 2009
	Scleractinian corals (*Porites lutea*)	ITS2 rRNA	*Symbiodinium*, *Cladocopium*, *Durusdinium*	Tan et al. 2020

Malaysia	Scleractinian corals (*Porites lutea*)	ITS2 rRNA	*Symbiodinium*, *Cladocopium*, *Durusdinium*	Tan et al. 2020
Thailand	Scleractinian corals, *Corallimorpharia* sp., sea anemones (Actiniidae & Stichodactyliidae), soft coral (Alcyonidae & Nephtheidae), gorgonian (*Gorgonia* sp.), giant clams (*Tridacna crocea*), Zoantharia (*Palythoa* sp.)	ITS1 rRNA, ITS2 rRNA, DGGE, microsatellite,	*Symbiodinium*, *Cladocopium*, *Durusdinium*, *Fugacium*, *Gerakladium*	LaJeunesse et al. 2010
Philippines	Giant clams (*Hippopus hippopus* & *Tridacna crocea*)	SSU 18S rRNA, RFLPs	*Symbiodinium*	Carlos et al. 1999
	Heliopora blue corals (*Heliopora coerulea*)	SSU 18S rRNA, RFLPs	*Cladocopium*	Taguba et al. 2016
	Scleractinian corals (*Acropora* spp.)	ITS2 rRNA, DGGE	*Cladocopium*	Ravelo and Conaco 2018
	Scleractinian corals	ITS2 rRNA, DGGE	*Cladocopium, Durusdinium*	Da-anoy et al. 2019
South China Sea	Scleractinian corals	LSU 28S rRNA	*Cladocopium*, *Durusdinium*	Tong et al. 2018
Timor-Leste	Scleractinian corals	mt cob, psbA^{ncr}	*Cladocopium*, *Durusdinium*	Brian et al. 2019

1