



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

Arief Pratomo<sup>1,2</sup>, Dietrich G. Bengen<sup>2</sup>, Neviaty P. Zamani<sup>2</sup>, Christopher Lane<sup>3</sup>, Austin T. Humphries<sup>4</sup>, Erin Borbee<sup>3</sup>, Beginer Subhan<sup>2</sup> and Hawis Madduppa<sup>2,5,†</sup>

<sup>1</sup>Raja Ali Haji Maritime University, Tanjungpinang, Indonesia

<sup>2</sup>Department of Marine Science and Technology, Institut Pertanian Bogor, Bogor, Indonesia

<sup>3</sup>Department of Biological Sciences, University of Rhode Island, Rhode Island, United States of America

<sup>4</sup>Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Rhode Island, United States of America

<sup>5</sup>Oceanogen Research Center, Bogor, Indonesia

† Deceased.

## 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  $\mu\text{m}$ . 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 *Hallaxium*. 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.

Submitted 6 October 2021  
Accepted 14 August 2022  
Published 24 October 2022

Corresponding author  
Beginer Subhan, [beginersubhan@apps.ipb.ac.id](mailto:beginersubhan@apps.ipb.ac.id)

Academic editor  
Xavier Pochon

Additional Information and  
Declarations can be found on  
page 20

DOI 10.7717/peerj.14006

© Copyright  
2022 Pratomo et al.

Distributed under  
Creative Commons CC-BY 4.0

## OPEN ACCESS

**Subjects** Biodiversity, Conservation Biology, Genetics, Marine Biology, Biological Oceanography  
**Keywords** Coral triangle, Coral hosts, 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., 2010a; LaJeunesse et al., 2010b; 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., 2010a; LaJeunesse et al., 2010b; LaJeunesse et al., 2018; 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), *Freudenthalidium* (clade Fr3), *Fugacium* (clade Fr5), *Gerakladium* (clade G), and *Hallaxium* (clade H) (LaJeunesse et al., 2018; LaJeunesse et al., 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, Cowlshaw & Wilson, 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, Cowlshaw & Wilson, 2006; Reimer & Todd, 2009; LaJeunesse et al., 2010a; LaJeunesse et al., 2010b; 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 marine 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 Abrar *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 Tables 1 and 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 h). Each sample was filtered twice using a peristaltic pump (Thermo Fisher Scientific) through 47 mm diameter polycarbonate membrane filters (Sterlitech) with two different pore sizes: 12  $\mu\text{m}$  first and then 0.4  $\mu\text{m}$ . According to Turner *et al.* (2014), a combination of  $\geq 0.2 \mu\text{m}$  filtration pore size and water volume enables optimal eDNA capture and maximize detection probability. In addition, a large pore size is required to avoid clogging the filters. The sediment samples, were shaken first and then

**Table 1** Coordinates of sampling stations at each coastal area around Lombok Island, Indonesia.

Coastal area	Station	Date	Depth (m) <sup>a</sup>	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.**

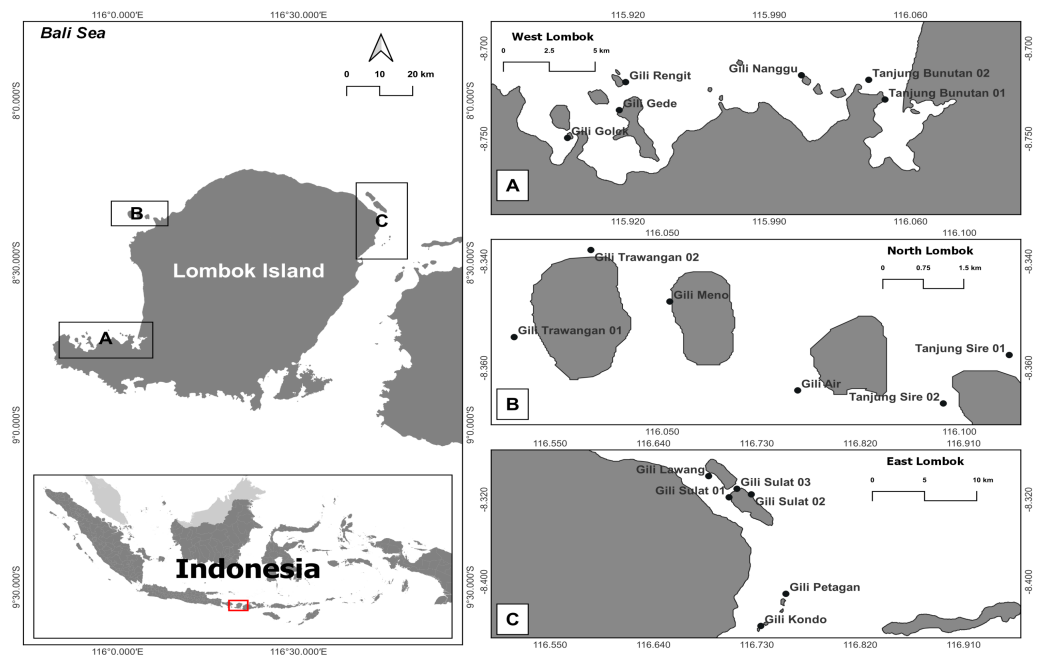
<sup>a</sup>In lowest low water level (LLWL) based on Hydrographic and Oceanographic Center, The Indonesian Navy (2007) and mean tidal range is 187 cm.

filtered 1–2 min after shaking. Each filter was cut into two, and each half was placed in a 1.5 mL vial prefilled with DNA Shield as a preservative. At the end of all eDNA survey activities, all the samples were transported to the Marine Biodiversity and Biosystematics Laboratory at Bogor Agricultural (IPB) University, Indonesia, via commercial courier and then stored at  $-20^{\circ}\text{C}$  until DNA extraction.

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 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 EB (130/E5/E5.4/SIP/2019), CL (461/SIP/FRP/E5/Dit.KI/XII/2017), and AH (455/SIP/FRP/E5/Dit.KI/XII/2017).

### 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, 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



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

Full-size [DOI: 10.7717/peerj.14006/fig-1](https://doi.org/10.7717/peerj.14006/fig-1)

chain reaction (PCR) platform and prepared for  $2 \times 250$  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  $\mu$ L of PCR reaction comprised 25  $\mu$ L of MyTM HS red mix (Bioline Ltd., London, UK), 1  $\mu$ L of (10  $\mu$ M) forward primer, 1  $\mu$ L of (10  $\mu$ M) reverse primer, and 1  $\mu$ L of DNA template. The final volume was adjusted to 49  $\mu$ L using ddH<sub>2</sub>O. 1x reaction was 0.2  $\mu$ M. The PCR product was checked via the electrophoresis final master mix concentration in 1  $\times$  reaction was 0.8  $\times$ , and the final primer concentration in of 5  $\mu$ L 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. The presence and length (bp) of the PCR product or amplicon were tested by electrophoresis. Successful amplicons were then purified using paramagnetic Kapa pure beads (bead-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

**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; bold font indicates Symbiodiniaceae were detected.

Location	Station	Seawater fraction		Sediment fraction	
		0.4–12 $\mu\text{m}$	>12 $\mu\text{m}$	0.4–12 $\mu\text{m}$	>12 $\mu\text{m}$
East Lombok	Gili Sulat 1	n.a.	EB356	<b>EB357</b>	<b>EB358</b>
	Gili Lawang	<b>EB367</b>	EB368	<b>EB369</b>	EB370
	Gili Sulat 2	EB359	EB360	EB361	<b>EB362</b>
	Gili Sulat 3	EB363	<b>EB364</b>	<b>EB365</b>	<b>EB366</b>
	Gili Petagan	n.a.	EB371	<b>EB372</b>	<b>EB373</b>
	Gili Kondo	n.a.	<b>EB374</b>	<b>EB375</b>	<b>EB376</b>
West Lombok	Gili Nanggu	n.a.	n.a.	<b>EB377</b>	n.a.
	Gili Rengit	n.a.	n.a.	<b>EB379</b>	n.a.
	Gili Golek	n.a.	n.a.	EB380	EB381
	Gili Gede	EB382	n.a.	EB383	n.a.
	Bunutan 1	n.a.	n.a.	<b>EB378</b>	n.a.
	Bunutan 2	n.a.	n.a.	n.a.	n.a.
North Lombok	Gili Trawangan 1	EB384	EB385	<b>EB386</b>	EB387
	Gili Air	<b>EB396</b>	n.a.	n.a.	n.a.
	Gili Trawangan 2	<b>EB388</b>	<b>EB389</b>	<b>EB390</b>	EB391
	Gili Meno	<b>EB392</b>	EB393	<b>EB394</b>	<b>EB395</b>
	Tanjung Sire 1	n.a.	n.a.	n.a.	n.a.
	Tanjung Sire 2	n.a.	n.a.	n.a.	n.a.

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). After quality checking, only 41 out of 72 samples were found to be of sufficiently high quality for sequencing (Table 2). The low quality of some libraries may be due to eDNA degradation during sample transport and extraction.

### Data processing and bioinformatic analyses

The obtained forward and reverse raw sequence data 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 analysis step. Cutadapt v.1.18 (Martin, 2011) was used to trim the reverse and forward primer sequences and remove short reads with lengths <100 bp and low quality reads with a Phred Q score of <20. Qiime2.2019.10 pipeline (Caporaso et al., 2010; Bolyen et al., 2019) was employed for further data processing. DADA2 v.2018.11.0 (Callahan et al., 2016) (via q2-dada2) was applied for denoising, joining denoised paired-end reads, filtering out chimeric sequences and singletons, and dereplicating sequences to produce amplicon sequence variants (ASVs). Owing to the high quality of the sequences obtained after Cutadapt procedure, trimming and truncating were not performed during DADA2 processing.



## ASV identification

Symbiodiniaceae species were identified from the eDNA sequences by classifying all ASVs (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% OTU reference sequences (<https://www.arb-silva.de/download/archive/qiime/>). Stoeck *et al.* (2010) showed the differential increase in diversity detected when the V9 dataset is clustered at 97%, 98%, 99%, and 100% sequence similarity for the minimum expected error rate. Putative Symbiodiniaceae ASVs were then filtered from the obtained eukaryote taxonomy table (File S2) (Table 3) and then assessed using the NCBI BLAST routine by selecting the best hit at >95% identity in the nr/nt database of NCBI (<https://www.ncbi.nlm.nih.gov/>, accessed on 1/19/2020, version 2.11.0). The BLAST results (File S3) were evaluated, and reference sequences (accessions) were selected for further analyses. Additional SSU 18S Symbiodiniaceae reference sequences (accessions) representing several families in the 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, Cowlshaw & Wilson (2006). The final compiled reference sequence database (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 1,000 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 (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

**Table 3** 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.

Methods → DB Ref.* →	Fit-Classifier-Naïve Bayes		BLAST			Phylogenetics	
	SILVA (97&99%)	Conf. rates**	Accession no.	NCBI <sup>d</sup>	%Id.***	Genera/subclades	Scores: SH-rlrt/lbt/ abayes/ufboot
OTUs							
OTU.sym1	Symbiodinium	0.999410455	KC816641.1	<i>Cladocopium</i> sp. clade C	100	<i>Cladocopium</i> /C.sym1	93.2/90.8/1/69
OTU.sym2	Symbiodinium	0.823380237	AY165766.1	<i>Symbiodinium</i> sp. ex <i>P. briareum</i> /D1 <sup>b</sup>	100	<i>Durusdinium</i> /D1.sym2	84.5/79.8/0.875/62
OTU.sym3	Symbiodinium	0.890746449	EF526860.1	uncultured marine Eukaryote	99.24	Unclassified Suessi-aceae/OTU.sym3	100/100/1/100
OTU.sym4	Symbiodinium	0.78273645	MH702366	CladeG2_V9_7221 <sup>c</sup>		Formaninifera Clade G/. G2.sym4	54.8/64.4/0.838/63
OTU.sym5	Symbiodinium	0.768964536	AB085912.1	<i>Cladocopium</i> sp. <sup>c</sup>		<i>Cladocopium</i> / C.sym5	93.2/90.8/1/69
OTU.sym6	Symbiodinium	0.993577182	AF238261.1	<i>Symbiodinium</i> sp. clade E/D1 <sup>b</sup>	99.24	<i>Durusdinium</i> / D1.sym6	84.5/79.8/0.875/62
OTU.sym7	Symbiodinium	0.995164623	KC816641.1	<i>Cladocopium</i> sp. clade C	99.24	<i>Cladocopium</i> /C.sym7	93.2/90.8/1/69
OTU.sym8	Symbiodinium	0.999188819	AF238258.1	<i>Symbiodinium</i> sp. type C	99.24	<i>Cladocopium</i> /C.sym8	93.2/90.8/1/69
OTU.sym9	Symbiodinium	0.82770918	KP404862.1	uncultured Eukaryote <sup>c</sup>		Unclassified Suessi-aceae/OTU.sym9	94.9/96.6/1/91
OTU.sym10	Symbiodinium	0.925078226	AB085912.1	<i>Cladocopium</i> sp. <sup>c</sup>		<i>Cladocopium</i> /C.sym10	93.2/90.8/1/69
OTU.sym11	Symbiodinium <sup>a</sup>	0.998952834	LN898222.1	<i>Yihiella yeosuensis</i>	100	<i>Yihiella</i> /OTU.sym11	83.5/80.5/0.963/79
OTU.sym12	Symbiodinium	0.991677932	MH702343.1	<i>Symbiodinium</i> sp. clade H	97.71	<i>Halluxium</i> /H.sym12	89.2/87/0.964/83
OTU.sym13	Symbiodinium <sup>a</sup>	0.942758624	LN898222.1	<i>Yihiella yeosuensis</i>	99.24	<i>Yihiella</i> /OTU.sym13	83.5/80.5/0.963/79
OTU.sym14	Symbiodinium	0.890990315		Incertae Sedis		Unclassified Suessi-aceae/OTU.sym14	68/62.4/0.43/91
OTU.sym15	Symbiodinium	0.999046044	KC816642.1	<i>Cladocopium</i> sp. clade C	99.24	<i>Cladocopium</i> /C.sym15	93.2/90.8/1/69
OTU.sym16	Symbiodinium	0.999527293	HM067612.1	<i>Symbiodinium</i> sp. 2-125/CladeC <sup>b</sup>	99.24	<i>Cladocopium</i> /C.sym16	93.2/90.8/1/69
OTU.sym17	Symbiodinium	0.987978975	MMETSP1371	<i>Symbiodinium</i> C15 <sup>c</sup>		<i>Cladocopium</i> /C.sym17	93.2/90.8/1/69
OTU.sym18	Symbiodinium	0.997997906	LK934670.1	<i>Breviolum minutum</i>	99.24	<i>Breviolum</i> /B.sym18	100/100/1/99
OTU.sym19	Symbiodinium	0.994027696	AF238261.1	<i>Symbiodinium</i> sp. clade E/D1 <sup>b</sup>	99.24	<i>Durusdinium</i> /D1.sym19	84.5/79.8/0.875/62

(continued on next page)



Table 3 (continued)

Methods → DB Ref.* →	Fit-Classifier-Naïve Bayes		BLAST			Phylogenetics	
	SILVA (97&99%)	Conf. rates**	Accession no.	NCBI <sup>d</sup>	%Id.***	Genera/subclades	Scores: SH-ahlr/lbt/ abayes/ufboot
<b>OTUs</b>							
OTU.sym20	Symbiodinium	0.742593275	<a href="#">LC361448.1</a>	<i>Ansanella natalensis</i>	98.47	<i>Ansanella</i> /OTU.sym20	26.8/54.4/0.465/52
OTU.sym21	Symbiodinium	0.978735399	<a href="#">AB085913.1</a>	<i>Cladocopium</i> sp. <sup>c</sup>		<i>Symbiodinium</i> /A.sym21	100/100/1/100
OTU.sym22	Symbiodinium	0.977772371	<a href="#">AY165766.1</a>	<i>Symbiodinium</i> sp. ex <i>P. briareum</i> /D1 <sup>b</sup>	100	<i>Durusdinium</i> /D1.sym22	84.5/79.8/0.875/62

**Notes.**

<sup>a</sup>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.

<sup>b</sup>According to [Decelle et al. \(2018\)](#).

<sup>c</sup>Non BLAST result.

<sup>d</sup>Nearest subclade branch (see [Fig. 3](#)).

\*Reference database.

\*\*Confidence level.

\*\*\*Percentage identity.

entropy) and beta diversity (Bray–Curtis dissimilarity) were estimated using q2-diversity after the samples were rarefied (subsampling 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

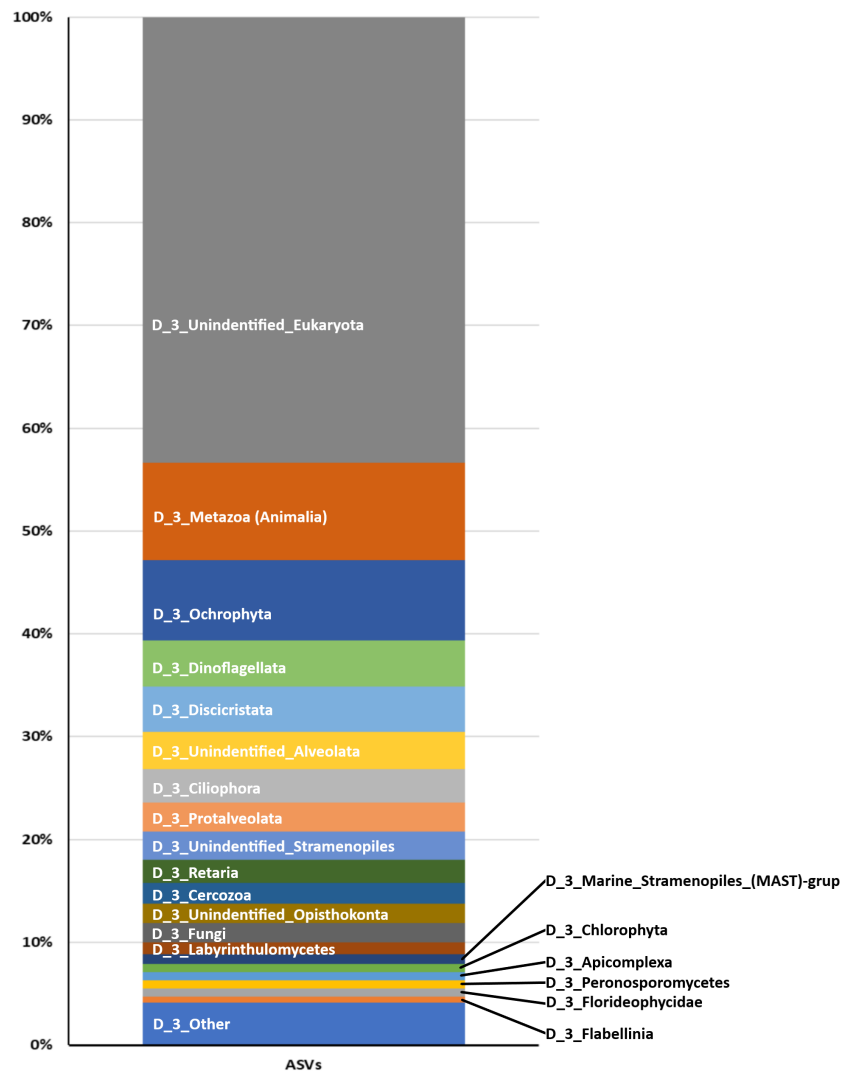
### Obtained sequences, ASVs, and eukaryote classification

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

### Symbiodiniaceae detection and classification

Table 3 summarizes the results of Symbiodiniaceae classification performed using a Eukaryote classifier (File S2) and BLAST (File S3) and phylogenetic analyses. The probabilistic classifier detected and classified the Symbiodiniaceae taxa at the family level. Twenty-two ASVs (named OTU.sym1 to OTU.sym22) were found to be putative Symbiodiniaceae with confidence levels ranging 0.743–0.999 (Table 3). BLAST results indicated that some ASVs were neither Symbiodiniaceae nor classified at the genus level. A partial phylogenetic reconstruction of the families in order Suessiales was conducted using the reference sequences obtained from the searched databases (File S4) and the putative Symbiodiniaceae ASV sequences from the study (Fig. 3A). Only 16 out of the 22 ASVs were identified as members of the monophyletic group of the Symbiodiniaceae family clade on the basis of the score of 100/100/1/99 for SH-mlrt/lbt/abayes/ufboot. Three of the six remaining ASVs were categorized in the clades representing genera in Family Suessiaceae, two ASVs were in the *Yihiella* clade (OTU.sym11 and OTU.sym13), and one was in the *Ansanella* clade (OTU.sym20). The remaining three ASVs were designated to the Suessiaceae family but were not classified at the 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)*. One ASV was allocated to each of clades *Symbiodinium* (A.sym21), *Breviolum* (B.sym18), Foraminifera Clade G (G2.sym4), and *Halluxium* (H.sym12). Eight ASVs were designated to *Cladocopium* (C.sym1, C.sym5, C.sym7, C.sym8, C.sym10, C.sym15, C.sym16, and C.sym17), and four ASVs allocated to *Durusdinium* (D1.sym2, D1.sym6, D1.sym19, and D1.sym22).

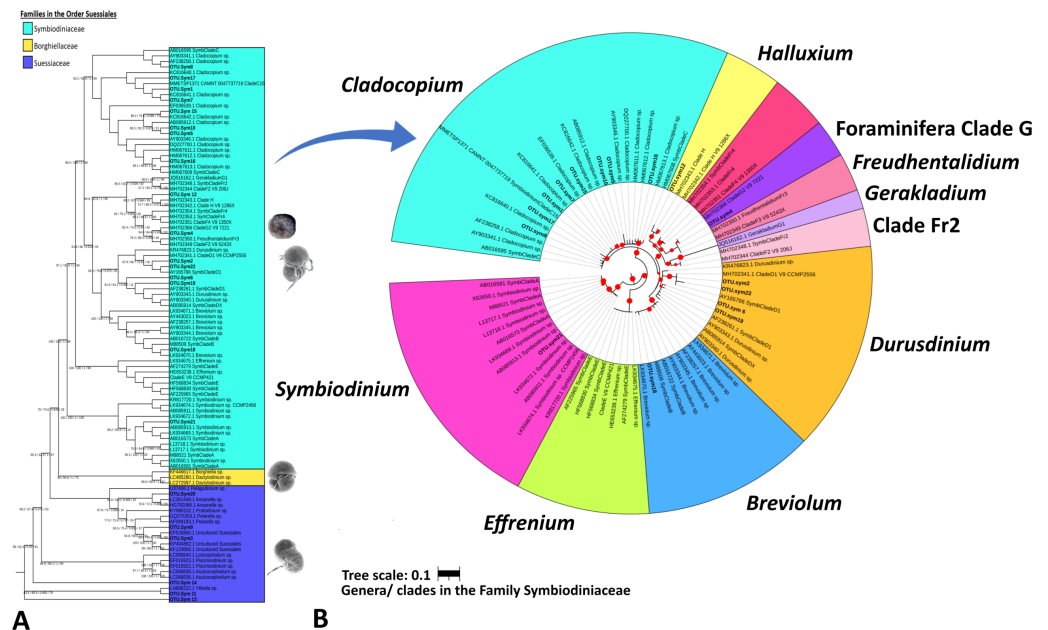


**Figure 2** Proportion of Eukaryote taxa. Based on the total ASVs of taxon level 4 out of 15 taxon levels according to the SILVA database (<https://www.arb-silva.de/>).

Full-size DOI: 10.7717/peerj.14006/fig-2

## 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 File S6). The presence/absence table shows the Symbiodiniaceae proportion per subclade by site–sample type–filter pore size combination (Table 4 and 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



**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-*alt*/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-*alt*. Phylogenetic reconstruction was performed in IQ-TREE and visualized with iTOL (<https://itol.embl.de/>).

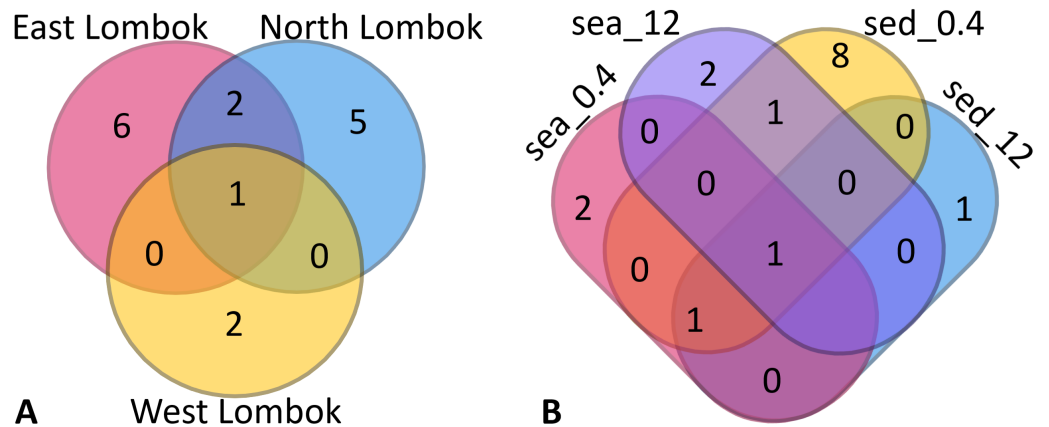
Full-size DOI: [10.7717/peerj.14006/fig-3](https://doi.org/10.7717/peerj.14006/fig-3)

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 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, Cowlshaw & Wilson, 2006](#); [Barneah et al., 2007](#); [LaJeunesse et al., 2010a](#); [LaJeunesse et al., 2010b](#); [LaJeunesse et al., 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

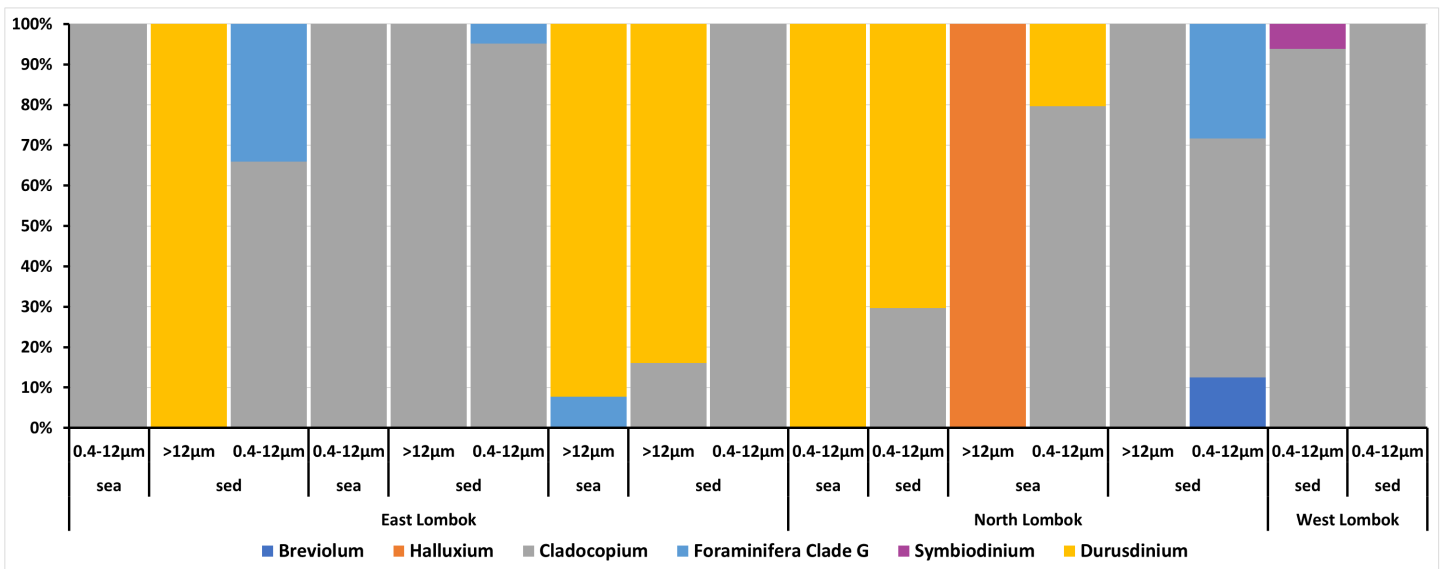


**Figure 4** Venn diagram of Symbiodiniaceae subclades around Lombok by: (A) coastal area and (B) method (sample type–filter pore size combination). Sample labels: sea = seawater sample; sed = sediment sample; \_0.4 and \_12 indicate the pore size of the filter (in  $\mu\text{m}$ ).

Full-size DOI: 10.7717/peerj.14006/fig-4

**Table 4** 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  $\geq 33.33\%$  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  $\mu\text{m}$ ; ESea12: East Lombok\_Sea Water\_>12  $\mu\text{m}$ ; ESed0.4: East Lombok\_Sediment\_0.4–12  $\mu\text{m}$ ; ESed12: East Lombok\_Sediment\_>12  $\mu\text{m}$ ; NSea0.4: North Lombok\_Sea Water\_0.4–12  $\mu\text{m}$ ; NSea12: North Lombok\_Sea Water\_>12  $\mu\text{m}$ ; NSed0.4: North Lombok\_Sediment\_0.4–12  $\mu\text{m}$ ; NSed12: North Lombok\_Sediment\_>12  $\mu\text{m}$ ; WSea0.4: West Lombok\_Sea Water\_0.4–12  $\mu\text{m}$ ; WSed0.4: West Lombok\_Sediment\_0.4–12  $\mu\text{m}$ .

Intersection inter site-sample type-fraction	Proportion per subclade (%)	Total	Subclades	Type
ESea0.4/ESed0.4/ESed12/NSea0.4/NSed0.4/NSed12/WSea0.4	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
WSea0.4	11.11	2	A.sym21	Unique
	11.11		C.sym5	Unique



**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.

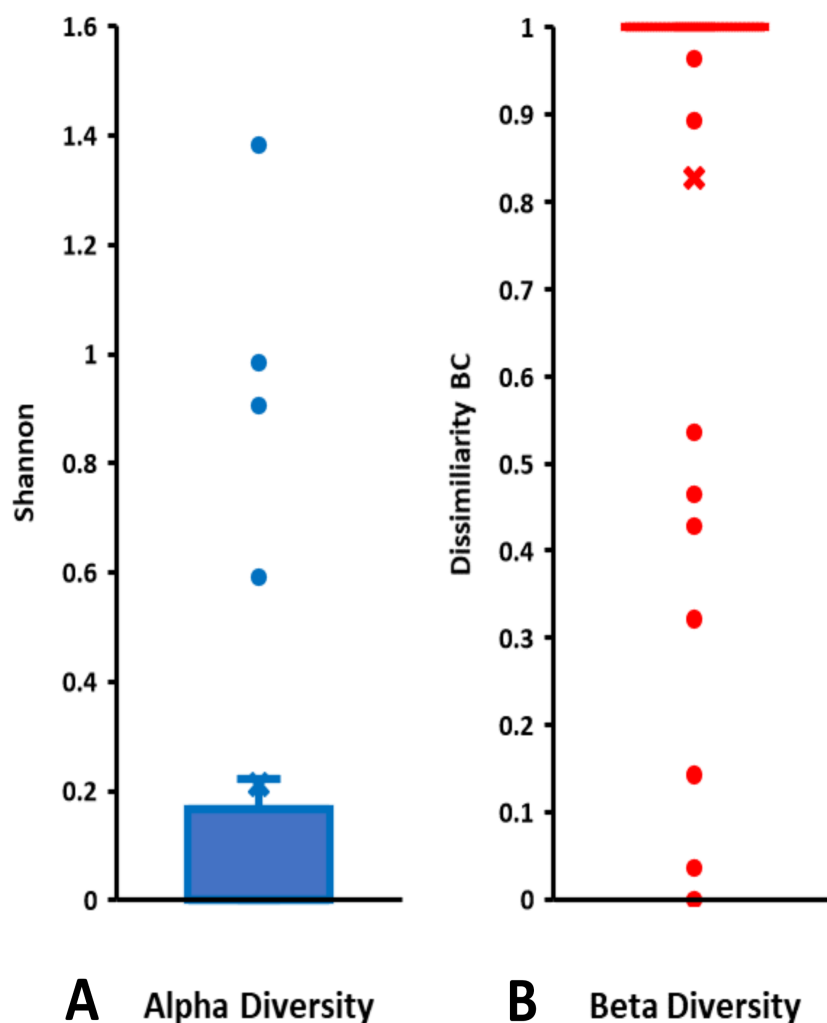
Full-size [DOI: 10.7717/peerj.14006/fig-5](https://doi.org/10.7717/peerj.14006/fig-5)

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, Cowlshaw & 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 comparative 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





**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. Cross symbols indicate the mean, and circles denote the outliers.

Full-size DOI: 10.7717/peerj.14006/fig-6

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, Parkinson & Trench (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, Cowlishaw & Wilson, 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., 2010a; LaJeunesse et al., 2010b; Rouzé et al., 2017*). The main coral endosymbionts, especially in Indo-Pacific, are species of *Cladocopium* and *Durusdinium*; meanwhile members *Symbiodinium* and *Breviolum* are common in corals in the Caribbean (*Baker, 2003; LaJeunesse et al., 2004; LaJeunesse et al., 2010a; LaJeunesse et al., 2010b; 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. goreau* (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

**Table 5** Comparison of Symbiodiniaceae studies in Indonesia and Southeast Asia region.

Geographic scope	Sample type(s)	Identification Method(s)	Genera	Reference(s)
<b>Indonesia:</b>				
Sulawesi	Sea slugs ( <i>Pteraeolidia ianthina</i> )	SSU 18S rRNA, LSU 28S rRNA, Single Stranded-Conformational Polymorphism (SSCP)	<i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Loh, Cowlshaw &amp; Wilson (2006)</a>
West Sumatra	Anthipatharian black corals ( <i>Cirrihipathes</i> sp.)	ITS2 rRNA, LSU 28S rRNA, denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphisms (RFLPs)	<i>Gerakladium</i>	<a href="#">Bo et al. (2011)</a>
Papua & West Papua	Giant clams ( <i>Tridacna</i> spp.)	ITS2 rRNA, DGGE	<i>Symbiodinium</i> , <i>Breviolum</i> , <i>Cladocopium</i>	<a href="#">DeBoer et al. (2012)</a>
Central Java	Scleractinian corals, sea anemones, <i>Tridacna</i> sp.	SSU 18S rRNA, RFLPs	<i>Symbiodinium</i> , <i>Breviolum</i> , <i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Purnomo (2014)</a>
West Nusa Tenggara	Seawater, sediment	V9-SSU 18S rRNA	<i>Symbiodinium</i> , <i>Breviolum</i> , <i>Cladocopium</i> , <i>Durusdinium</i> , <i>Gerakladium</i> , <i>Halluxium</i>	This Study
<b>Southeast Asia:</b>				
Palau	Sponges (porifera), giant clams ( <i>Tridacna</i> spp.), other bivalves (cardiids), foraminifera ( <i>Amphisorus hemprichii</i> )	SSU 18S rRNA, RFLPs	<i>Symbiodinium</i> , <i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Carlos et al. (1999)</a>
	Scleractinian corals	ITS1 rRNA, SSCP	<i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Fabricius et al. (2004)</a>
	Scleractinian corals ( <i>Porites cylindrica</i> )	ITS2 rRNA, psbA <sup>ncr</sup>	<i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Kurihara et al. (2021)</a>
Singapore	Sea slugs ( <i>Pteraeolidia ianthina</i> )	SSU 18S rRNA, LSU 28S rRNA, SSCP	<i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Loh, Cowlshaw &amp; Wilson (2006)</a>
	Zoantharians	mt 16S rRNA, mt COI, ITS rRNA	<i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Reimer &amp; Todd (2009)</a>
	Scleractinian corals ( <i>Porites lutea</i> )	ITS2 rRNA	<i>Symbiodinium</i> , <i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Tan et al. (2020)</a>
Malaysia	Scleractinian corals ( <i>Porites lutea</i> )	ITS2 rRNA	<i>Symbiodinium</i> , <i>Cladocopium</i> , <i>Durusdinium</i>	<a href="#">Tan et al. (2020)</a>
Thailand	Scleractinian corals, <i>Corallimorpharia</i> sp., sea anemones (Actiniidae & Stichodactyliidae), soft coral (Alcyonidae & Nephtheidae), gorgonian ( <i>Gorgonia</i> sp.), giant clams ( <i>Tridacna crocea</i> ), Zoantharia ( <i>Palythoa</i> sp.)	ITS1 rRNA, ITS2 rRNA, DGGE, microsatellite,	<i>Symbiodinium</i> , <i>Cladocopium</i> , <i>Durusdinium</i> , <i>Fugacium</i> , <i>Gerakladium</i>	<a href="#">LaJeunesse et al. (2010a)</a> and <a href="#">LaJeunesse et al. (2010b)</a>

(continued on next page)

Table 5 (continued)

Geographic scope	Sample type(s)	Identification Method(s)	Genera	Reference(s)
Philippines	Giant clams ( <i>Hippopus hippopus</i> & <i>Tridacna crocea</i> )	SSU 18S rRNA, RFLPs	<i>Symbiodinium</i>	<i>Carlos et al. (1999)</i>
	<i>Heliopora</i> blue corals ( <i>Heliopora coerulea</i> )	SSU 18S rRNA, RFLPs	<i>Cladocopium</i>	<i>Taguba, Sotto &amp; Geraldino (2016)</i>
	Scleractinian corals ( <i>Acropora</i> spp.)	ITS2 rRNA, DGGE	<i>Cladocopium</i>	<i>Ravelo &amp; Conaco (2018)</i>
	Scleractinian corals	ITS2 rRNA, DGGE	<i>Cladocopium</i> , <i>Durusdinium</i>	<i>Da-Anoy, Cabaitan &amp; Conaco (2019)</i>
South China Sea	Scleractinian corals	LSU 28S rRNA	<i>Cladocopium</i> , <i>Durusdinium</i>	<i>Tong et al. (2018)</i>
Timor-Leste	Scleractinian corals	mt cob, psbA <sup>ncr</sup>	<i>Cladocopium</i> , <i>Durusdinium</i>	<i>Brian, Davy &amp; Wilkinson (2019)</i>

(*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; Natsir, 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 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. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors: 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 there are no competing interests.

### Author Contributions

- Arief Pratomo conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Dietrich G. Bengen analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Neviaty P. Zamani analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Christopher Lane conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Austin T. Humphries conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Erin Borbee conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.



- Beginer Subhan performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Hawis Madduppa conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

### Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

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 EB (130/E5/E5.4/SIP/2019), CL and AH (455/SIP/FRP/E5/Dit.KI/XII/2017).

### Data Availability

The following information was supplied regarding data availability:

The raw sequence data is available at GenBank: [PRJNA768103](https://www.ncbi.nlm.nih.gov/nuclseq/PRJNA768103).

The outative Symbiodiniaceae OTU sequence data is available at GenBank: [SRP339775](https://www.ncbi.nlm.nih.gov/nuclseq/SRP339775).

The code for this research analyses is available at GitHub: [https://github.com/ariief2021/Symbio\\_Qiime2.git](https://github.com/ariief2021/Symbio_Qiime2.git).

### Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.14006#supplemental-information>.

## REFERENCES

- Abrego D, Ulstrup KE, Willis BL, Van Oppen MJH. 2008.** Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences* **275**:2273–2282 DOI [10.1098/rspb.2008.0180](https://doi.org/10.1098/rspb.2008.0180).
- Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM. 2009.** A method for studying protistan diversity using massively parallel sequencing of V9 hyper-variable regions of small-subunit ribosomal RNA genes. *PLOS ONE* **4**(7):e6372 DOI [10.1371/journal.pone.0006372](https://doi.org/10.1371/journal.pone.0006372).
- Anderson MJ. 2001.** A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**:32–46.
- Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, LaJeunesse TC, Voolstra CR. 2014.** Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. *Molecular Ecology* **23**:4418–4433 DOI [10.1111/mec.12869](https://doi.org/10.1111/mec.12869).

- Auliaherliaty L, Dewi KT, Priohandono YA. 2004.** Foraminifera Di Teluk Sepi—Blongas, Lombok Selatan, Nusa Tenggara Barat Dan Kaitannya Dengan Faktor Lingkungan. *Jurnal Geologi Kelautan* 2:1–8  
[DOI 10.32693/jgk.2.3.2004.115](https://doi.org/10.32693/jgk.2.3.2004.115).
- Baker AC. 2003.** Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics* 34:661–689 [DOI 10.1146/annurev.ecolsys.34.011802.132417](https://doi.org/10.1146/annurev.ecolsys.34.011802.132417).
- Bálint M, Nowak C, Márton O, Pauls SU, Wittwer C, Aramayo JL, Schulze A, Chambert T, Cocchiararo B, Jansen M. 2018.** Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources* 18:1415–1426 [DOI 10.1111/1755-0998.12934](https://doi.org/10.1111/1755-0998.12934).
- Barneah O, Brickner I, Hooge M, Weis VM, LaJeunesse TC, Benayahu Y. 2007.** Three party symbiosis: acoelomorph worms, corals and unicellular algal symbionts in Eilat (Red Sea). *Marine Biology* 151:1215–1223 [DOI 10.1007/s00227-006-0563-2](https://doi.org/10.1007/s00227-006-0563-2).
- Barnes MA, Turner CR. 2016.** The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17:1–17  
[DOI 10.1007/s10592-015-0775-4](https://doi.org/10.1007/s10592-015-0775-4).
- Baskett ML, Gaines SD, Nisbet RM. 2009.** Symbiont diversity may help coral reefs survive moderate climate change. *Ecological Applications* 19:3–17  
[DOI 10.1890/08-0139.1](https://doi.org/10.1890/08-0139.1).
- Bay LK, Doyle J, Logan M, Berkelmans R. 2016.** Recovery from bleaching is mediated by threshold densities of background thermo-tolerant symbiont types in a reef-building coral. *Royal Society Open Science* 3(6):160322 [DOI 10.1098/rsos.160322](https://doi.org/10.1098/rsos.160322).
- Berkelmans R, Van Oppen MJH. 2006.** The role of zooxanthellae in the thermal tolerance of corals: a nugget of hope for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences* 273(1599):2305–2312  
[DOI 10.1098/rspb.2006.3567](https://doi.org/10.1098/rspb.2006.3567).
- Bo M, Baker AC, Gaino E, Wirshing HH, Scoccia F, Bavestrello G. 2011.** First description of algal mutualistic endosymbiosis in a black coral (Anthozoa: Antipatharia). *Marine Ecology Progress Series* 435:1–11 [DOI 10.3354/meps09228](https://doi.org/10.3354/meps09228).
- Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JGregory. 2018.** Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:1–17  
[DOI 10.1186/s40168-018-0470-z](https://doi.org/10.1186/s40168-018-0470-z).
- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Bin KK, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciulek T, Kreps J, Langille MGI, Lee J, Ley RE, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD,**

- McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, Van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, Von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37:852–857 DOI 10.1038/s41587-019-0209-9.
- Bongaerts P, Carmichael M, Hay KB, Tonk L, Frade PR, Hoegh-Guldberg O. 2015. Prevalent endosymbiont zonation shapes the depth distributions of scleractinian coral species. *Royal Society Open Science* 2:140297–140297 DOI 10.1098/rsos.140297.
- Bourne DG, Morrow KM, Webster NS. 2016. Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annual Review of Microbiology* 70:317–340 DOI 10.1146/annurev-micro-102215-095440.
- Brian JI, Davy SK, Wilkinson SP. 2019. Elevated Symbiodiniaceae richness at Atauro Island (Timor-Leste): a highly biodiverse reef system. *Coral Reefs* 38:123–136 DOI 10.1007/s00338-018-01762-9.
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–583 DOI 10.1038/nmeth.3869.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335–336 DOI 10.1038/nmeth.f.303.
- Carlos AA, Baillie BK, Kawachi M, Maruyama T. 1999. Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. *Journal of Phycology* 35:1054–1062 DOI 10.1046/j.1529-8817.1999.3551054.x.
- Castro-Sanguino C, Sánchez JA. 2012. Dispersal of *Symbiodinium* by the stoplight parrotfish *Sparisoma viride*. *Biology Letters* 8:282–286 DOI 10.1098/rsbl.2011.0836.
- Clair DC, Starko S, Tietjen KL, Epstein HE, Cunning R, Cobb KM, Baker AC, Gates RD, Baum JK. 2020. Dynamic symbioses reveal pathways to coral survival through prolonged heatwaves. *Nature Communications* 11:1–10 DOI 10.1038/s41467-020-19169-y.
- Coffroth MA, Lewis CF, Santos SR, Weaver JL. 2006. Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians. *Current Biology* 16:985–987 DOI 10.1016/j.cub.2006.10.049.

- 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(1809)**:20141725 DOI [10.1098/rspb.2014.1725](https://doi.org/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](https://doi.org/10.1017/S0025315419000158).
- Davy SK, Allemand D, Weis VM. 2012.** Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews* **76**:229–261 DOI [10.1128/MMBR.05014-11](https://doi.org/10.1128/MMBR.05014-11).
- DeBoer TS, Baker AC, Erdmann MV, Ambariyanto O, 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](https://doi.org/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](https://doi.org/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 metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology* **26**:5872–5895 DOI [10.1111/mec.14350](https://doi.org/10.1111/mec.14350).
- Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011.** Persistence of environmental DNA in freshwater ecosystems. *PLOS ONE* **6**:8–11 DOI [10.1371/journal.pone.0023398](https://doi.org/10.1371/journal.pone.0023398).
- Dewi KT, Arifin L, Yuningsih A, Permanawati Y. 2012.** Meiofauna (Foraminifera) in sediments and its relation to white sandy beach of Senggigi and water condition off West Lombok. *Jurnal Ilmu dan Teknologi Kelautan Tropis* **4**:47–54 DOI [10.29244/jitkt.v4i1.7805](https://doi.org/10.29244/jitkt.v4i1.7805).
- Fabricius KE, Mieog JC, Colin PL, Idip D, Van Oppen MJH. 2004.** Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology* **13**:2445–2458 DOI [10.1111/j.1365-294X.2004.02230.x](https://doi.org/10.1111/j.1365-294X.2004.02230.x).
- Freudenthal HD. 1962.** *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov. a zooxanthella: taxonomy, life cycle, and morphology. *Protozoology* **9**:45–52 DOI [10.1111/j.1550-7408.1962.tb02579.x](https://doi.org/10.1111/j.1550-7408.1962.tb02579.x).
- Fujise L, Suggett DJ, Stat M, Kahlke T, Bunce M, Gardner SG, Goyen S, Woodcock S, Ralph PJ, Seymour JR, Siboni N, Nitschke MR. 2021.** Unlocking the phylogenetic diversity, primary habitats, and abundances of free-living Symbiodiniaceae on a coral reef. *Molecular Ecology* **30**:343–360 DOI [10.1111/mec.15719](https://doi.org/10.1111/mec.15719).
- Fujise L, Yamashita H, Suzuki G, Sasaki K, Liao LM, Koike K. 2014.** Moderate thermal stress causes active and immediate expulsion of photosynthetically

- damaged zooxanthellae (*Symbiodinium*) from corals. *PLOS ONE* **9**:1–18  
[DOI 10.1371/journal.pone.0114321](https://doi.org/10.1371/journal.pone.0114321).
- Gelis ERE, Kamal MM, Subhan B, Bachtiar I, Sani LMI, Madduppa H. 2021.** Environmental biomonitoring of reef fish community structure with eDNA metabarcoding in the Coral Triangle. *Environmental Biology of Fishes* **104**:887–903  
[DOI 10.1007/s10641-021-01118-3](https://doi.org/10.1007/s10641-021-01118-3).
- Giyanto Abrar M, Hadi TA, Budiyo A, Hafizt M, Salatalohy A, Iswari MY. 2017.** *Status terumbu karang di Indonesia 2017*. Jakarta: P2O-LIPI.
- Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P. 2016.** Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* **7**:1299–1307  
[DOI 10.1111/2041-210X.12595](https://doi.org/10.1111/2041-210X.12595).
- Grupstra CGB, Rabbitt KM, Howe-Kerr LI, Correa AMS. 2021.** Fish predation on corals promotes the dispersal of coral symbionts. *bioRxiv*  
[DOI 10.1101/2020.08.10.243857](https://doi.org/10.1101/2020.08.10.243857).
- Hirose M, Reimer JD, Hidaka M, Suda S. 2008.** Phylogenetic analyses of potentially free-living *Symbiodinium* spp. isolated from coral reef sand in Okinawa, Japan. *Marine Biology* **155**:105–112 [DOI 10.1007/s00227-008-1011-2](https://doi.org/10.1007/s00227-008-1011-2).
- Hoadley KD, Lewis AM, Wham DC, Pettay DT, Grasso C, Smith R, Kemp DW, LaJeunesse TC, Warner ME. 2019.** Host–symbiont combinations dictate the photo-physiological response of reef-building corals to thermal stress. *Scientific Reports* **9**:1–16 [DOI 10.1038/s41598-019-46412-4](https://doi.org/10.1038/s41598-019-46412-4).
- Howells EJ, Abrego D, Liew YJ, Burt JA, Meyer E, Aranda M. 2021.** Enhancing the heat tolerance of reef-building corals to future warming. *Science Advances* **7**:eabg6070  
[DOI 10.1126/sciadv.abg6070](https://doi.org/10.1126/sciadv.abg6070).
- Hume BCC, D’Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J. 2015.** *Symbiodinium thermophilum* sp. nov. a thermotolerant symbiotic alga prevalent in corals of the world’s hottest sea, the Persian/Arabian Gulf. *Scientific Reports* **5**:8562  
[DOI 10.1038/srep08562](https://doi.org/10.1038/srep08562).
- Jeong HJ, Lee SY, Kang NS, Yoo YD, Lim AS, Lee MJ, Kim HS, Yih W, Yamashita H, LaJeunesse TC. 2014.** Genetics and morphology characterize the dinoflagellate *Symbiodinium voratum*, n. sp. (dinophyceae) as the sole representative of *Symbiodinium* clade e. *Journal of Eukaryotic Microbiology* **61**:75–94 [DOI 10.1111/jeu.12088](https://doi.org/10.1111/jeu.12088).
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**:587–589 [DOI 10.1038/nmeth.4285](https://doi.org/10.1038/nmeth.4285).
- Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780 [DOI 10.1093/molbev/mst010](https://doi.org/10.1093/molbev/mst010).
- Kimes NE, Johnson WR, Torralba M, Nelson KE, Weil E, Morris PJ. 2013.** The *Montastraea faveolata* microbiome: ecological and temporal influences on a

- Caribbean reef-building coral in decline. *Environmental Microbiology* **15**:2082–2094 DOI [10.1111/1462-2920.12130](https://doi.org/10.1111/1462-2920.12130).
- Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013.** Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology* **79**:5112–5120 DOI [10.1128/AEM.01043-13](https://doi.org/10.1128/AEM.01043-13).
- Kruskal WH, Wallis WA. 1952.** Journal of the American use of ranks in one-criterion variance analysis. *Journal of The American Statistical Association* **47(260)**:37–41 DOI [10.1080/01621459.1952.10483441](https://doi.org/10.1080/01621459.1952.10483441).
- Kurihara H, Watanabe A, Tsugi A, Mimura I, Hongo C, Kawai T, Reimer JD, Kimoto K, Gouezo M, Golbuu Y. 2021.** Potential local adaptation of corals at acidified and warmed Nikko Bay, Palau. *Scientific Reports* **11**:1–11 DOI [10.1038/s41598-021-90614-8](https://doi.org/10.1038/s41598-021-90614-8).
- LaJeunesse TC. 2005.** Species radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution* **22**:570–581 DOI [10.1093/molbev/msi042](https://doi.org/10.1093/molbev/msi042).
- LaJeunesse TC. 2020.** Zooxanthellae. *Current Biology* **30(19)**:R1110–R1113 DOI [10.1016/j.cub.2020.03.058](https://doi.org/10.1016/j.cub.2020.03.058).
- LaJeunesse TC, Bhagooli R, Hidaka M, De Vantier L, Done T, Schmidt G, Fitt W, Hoegh-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 DOI [10.3354/meps284147](https://doi.org/10.3354/meps284147).
- 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](https://doi.org/10.1016/j.cub.2018.07.008).
- LaJeunesse TC, Parkinson JE, Trench RK. 2012.** Symbiodinium, Version 04 2012. Available at <http://tolweb.org/Symbiodinium/126705/2012.07.04>.
- LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O, Fitt WK. 2010a.** 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](https://doi.org/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. 2010b.** 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](https://doi.org/10.1098/rspb.2010.0385).
- LaJeunesse TC, Wiedenmann J, Casado-Amezúa P, D’Ambra I, Turnham KE, Nitschke MR, Oakley CA, Goffredo S, Spano CA, Cubillos VM, Davy SK, Suggett DJ. 2021.** Revival of *Philozoon Geddes* for host-specialized dinoflagellates, ‘zooxanthellae’, in animals from coastal temperate zones of northern and southern hemispheres.



- European Journal of Phycology* 57:166–180  
DOI 10.1080/09670262.2021.1914863.
- Lesser MP, Stat M, Gates RD. 2013.** The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs* 32:603–611  
DOI 10.1007/s00338-013-1051-z.
- Littman RA, Van Oppen MJH, Willis BL. 2008.** Methods for sampling free-living *Symbiodinium* (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef). *Journal of Experimental Marine Biology and Ecology* 364:48–53  
DOI 10.1016/j.jembe.2008.06.034.
- Loh WKW, Cowlshaw M, Wilson NG. 2006.** Diversity of *Symbiodinium* dinoflagellate symbionts from the Indo-Pacific sea slug *Pteraeolidia ianthina* (Gastropoda: Mollusca). *Marine Ecology Progress Series* 320:177–184  
DOI 10.3354/meps320177.
- Madduppa H, Cahyani NKD, Anggoro AW, Subhan B, Jefri E, Sani LMI, Arafat D, Akbar N, Bengen DG. 2021.** eDNA metabarcoding illuminates species diversity and composition of three phyla (chordata, mollusca and echinodermata) across Indonesian coral reefs. *Biodiversity and Conservation* 30:3087–3114  
DOI 10.1007/s10531-021-02237-0.
- Martin M. 2011.** Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet* 17(1):200 DOI 10.14806/ej.17.1.200.
- Minh B, Trifinopoulos J, Schrepf D, Schmidt HA. 2021.** IQ-TREE version 2.1.2: tutorials and manual phylogenomic software by maximum likelihood.
- Montresor M, Lovejoy C, Orsini L, Procaccini G, Roy S. 2003.** Bipolar distribution of the cyst-forming dinoflagellate *Polarella glacialis*. *Polar Biology* 26:186–194  
DOI 10.1007/s00300-002-0473-9.
- Natsir SM. 2009.** First record of agglutinated foraminifera from Lombok. *Journal of Coastal Development* 13:46–53.
- Natsir SM. 2010.** Distribusi foraminifera bentik resen di Perairan Lombok (suatu tinjauan di daerah Gili Air. *Gili Meno dan Gili Trawangan*). *Biosfera* 27:95–102  
DOI 10.20884/1.mib.2010.27.2.198.
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32:268–274 DOI 10.1093/molbev/msu300.
- Nitschke MR, Craveiro SC, Brandão C, Fidalgo C, Serôdio J, Calado AJ, Frommlet JC. 2020.** Description of *Freudenthalidium* gen. nov. and *Halluxium* gen. nov. to formally recognize Clades Fr3 and H as genera in the family Symbiodiniaceae (Dinophyceae). *Journal of Phycology* 56:923–940 DOI 10.1111/jpy.12999.
- Pandeirada MS, Craveiro SC, Calado AJ. 2013.** Freshwater dinoflagellates in Portugal (W Iberia): a critical checklist and new observations. *Nova Hedwigia* 97:321–348  
DOI 10.1127/0029-5035/2013/0119.

- Pochon X, Garcia-Cuetos L, Baker AC, Castella E, Pawlowski J. 2007.** One-year survey of a single Micronesian reef reveals extraordinarily rich diversity of *Symbiodinium* types in soritid foraminifera. *Coral Reefs* **26**:867–882 DOI [10.1007/s00338-007-0279-x](https://doi.org/10.1007/s00338-007-0279-x).
- Pochon X, Gates RD. 2010.** A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Molecular Phylogenetics and Evolution* **56**:492–497 DOI [10.1016/j.ympev.2010.03.040](https://doi.org/10.1016/j.ympev.2010.03.040).
- Pochon X, LaJeunesse TC. 2021.** Miliolidium n. gen, a new symbiodiniacean genus whose members associate with soritid foraminifera or are free-living. *Journal of Eukaryotic Microbiology* **68**(4):e12856 DOI [10.1111/jeu.12856](https://doi.org/10.1111/jeu.12856).
- Pochon X, LaJeunesse TC, Pawlowski J. 2004.** Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta). *Marine Biology* **146**:17–27 DOI [10.1007/s00227-004-1427-2](https://doi.org/10.1007/s00227-004-1427-2).
- Pochon X, Pawlowski J. 2006.** Review article Evolution of the soritids-*Symbiodinium* symbiosis. *Symbiosis* **42**:77–88.
- Pochon X, Pawlowski J, Zaninetti L, Rowan R. 2001.** High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. *Marine Biology* **139**:1069–1078 DOI [10.1007/s002270100674](https://doi.org/10.1007/s002270100674).
- Pochon X, Putnam HM, Gates RD. 2014.** Multi-gene analysis of *Symbiodinium* dinoflagellates: a perspective on rarity, symbiosis, and evolution. *PeerJ* **2**:e394 DOI [10.7717/peerj.394](https://doi.org/10.7717/peerj.394).
- Purnomo PW. 2014.** Translocation study of some zooxanthellae clade to the survival and growth of *Goniastrea aspera* after bleaching. *International Journal of Marine and Aquatic Resource Conservation and Co-existence* **1**:50–56 DOI [10.14710/ijfst.7.1.39-45](https://doi.org/10.14710/ijfst.7.1.39-45).
- Rajan V. 2012.** A method of alignment masking for refining the phylogenetic signal of multiple sequence alignments. *Molecular Biology and Evolution* **30**:689–712 DOI [10.1093/molbev/mss264](https://doi.org/10.1093/molbev/mss264).
- Ramsby BD, Hill MS, Thornhill DJ, Steenhuizen SF, Achlatis M, Lewis AM, LaJeunesse TC. 2017.** Sibling species of mutualistic *Symbiodinium* clade G from bioeroding sponges in the western Pacific and western Atlantic oceans. *Journal of Phycology* **53**:951–960 DOI [10.1111/jpy.12576](https://doi.org/10.1111/jpy.12576).
- Ravelo SF, Conaco C. 2018.** Comparison of the response of in hospite and ex hospite *Symbiodinium* to elevated temperature. *Marine and Freshwater Behaviour and Physiology* **51**:93–108 DOI [10.1080/10236244.2018.1503935](https://doi.org/10.1080/10236244.2018.1503935).
- Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014.** The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology* **51**:1450–1459 DOI [10.1111/1365-2664.12306](https://doi.org/10.1111/1365-2664.12306).
- Reimer JD, Todd P. 2009.** Preliminary molecular examination of zooxanthellate zoanthid (Hexacorallia, Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in Singapore. *Raffles Bulletin of Zoology* **22**:103–120.

- Rouzé H, Lecellier GJ, Saulnier D, Planes S, Gueguen Y, Wirshing HH, Berteaux-Lecellier V. 2017.** An updated assessment of *Symbiodinium* spp. that associate with common scleractinian corals from Moorea (French Polynesia) reveals high diversity among background symbionts and a novel finding of clade B. *PeerJ* 5:e2856 DOI 10.7717/peerj.2856.
- Rowan R, Powers DA. 1991.** A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. *Science* 251:1348–1351 DOI 10.1126/science.251.4999.1348.
- Sakata MK, Yamamoto S, Gotoh RO, Miya M, Yamanaka H, Minamoto T. 2020.** Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA. *Environmental DNA* 2:505–518 DOI 10.1002/edn3.75.
- Schönberg CHL, Loh WKW. 2005.** Molecular identity of the unique symbiotic dinoflagellates found in the bioeroding demosponge *Cliona orientalis*. *Marine Ecology Progress Series* 299:157–166 DOI 10.3354/meps299157.
- Shinzato C, Zayasu Y, Kanda M, Kawamitsu M, Satoh N, Yamashita H, Suzuki G. 2018.** Using seawater to document coral-zooxanthella diversity: a new approach to coral reef monitoring using environmental DNA. *Frontiers in Marine Science* 5:1–12 DOI 10.3389/fmars.2018.00028.
- Smart AS, Weeks AR, Van Rooyen AR, Moore A, McCarthy MA, Tingley R. 2016.** Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution* 7:1291–1298 DOI 10.1111/2041-210X.12598.
- Stat M, Gates RD. 2011.** Clade D *Symbiodinium* in scleractinian corals: a nugget of hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology* 2011:730715 DOI 10.1155/2011/730715.
- Stat M, Morris E, Gates RD. 2008.** Functional diversity in coral-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* 105:9256–9261 DOI 10.1073/pnas.0801328105.
- Stoeck T, Bass D, Nebel M, Christen R, Meredith D. 2010.** Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology* 19:21–31 DOI 10.1111/j.1365-294X.2009.04480.x.
- Strickler KM, Fremier AK, Goldberg CS. 2014.** Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation* 183:85–92 DOI 10.1016/j.biocon.2014.11.038.
- Suggett DJ, Warner ME, Leggat W. 2017.** Symbiotic dinoflagellate functional diversity 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](https://doi.org/10.1111/1365-2435.12694).
- Taguba CA, Sotto FB, Geraldino PJJ. 2016.** Identification of *Symbiodinium* clade of the blue coral *Heliopora Coerulea* (Pallas, 1766) (Helioporacea: Helioporidae) from surrounding waters in Central Visayas, Philippines. In: Dautova TN, ed. *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*. Kobe: Asia-Pacific Network for Global Change Research, 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](https://doi.org/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](https://doi.org/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**(8):e41732 DOI [10.1371/journal.pone.0041732](https://doi.org/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](https://doi.org/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](https://doi.org/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 microbial eDNA. *Methods in Ecology and Evolution* **5**:676–684 DOI [10.1111/2041-210X.12206](https://doi.org/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](https://doi.org/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](https://doi.org/10.1007/s00338-010-0666-6).
- Veron JEN, De Vantier 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](https://doi.org/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](https://doi.org/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](https://doi.org/10.1016/j.ympev.2021.107158).