

Genetic Identification of Symbiotic Algae (Symbiodiniaceae) in Coral Bleaching on Sambangan Island, Karimunjawa

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Abstract

Recently, there has been a global decline of coral reef ecosystems, primarily caused by major threats such as global warming. This phenomenon has a significant negative impact on coral reefs, including rising sea temperatures that trigger coral bleaching. Composition of symbiotic algae, Symbiodiniaceae, in corals determines how corals respond to environmental stress. This study aimed to investigate the community of the symbiotic algae during bleaching event by applying molecular identification technique. Coral bleaching samples from different genera were randomly collected from three different location around Sambangan Island, Karimunjawa at the depth of 1 – 2 m. DNA Barcoding of Internal Transcribed Spacer (ITS1, 5.8 rRNA and ITS2) gene were performed. Our result shows that four types of Symbiodiniaceae were closely related to the symbiotic algae found in this study, i.e. *Cladocopium goreaui*, *Cladocopium* sp., *Symbiodinium* sp. Clade C, and *Symbiodinium* sp. C1. The phylogenetic tree analysis showed that all the Symbiodiniaceae species found formed a single clade with a low genetic distance value. All of these species belong to the same genus *Cladocopium*. This type of symbiotic algae is known to have broad tolerance to temperature changes and other stress factors.

Keywords: Coral Bleaching; Symbiodiniaceae; DNA Barcoding; *Cladocopium*; Karimunjawa

Introduction

Coral reefs engage in a mutualistic symbiosis with photosynthetic algae from the Symbiodiniaceae family (Mansfield and Gilmore, 2019). These algae live inside the tissues of corals and supply most of the coral's energy through photosynthesis (McIlroy et al., 2022). In return, the Symbiodiniaceae living in coral tissue receive shelter and utilize nutrients such as nitrate, phosphate, and carbon dioxide produced by the coral's metabolic processes. Additionally, they contribute to the vibrant color of the coral (Susanto et al., 2013). Recently, the condition of coral reef ecosystems has declined globally due to various threats, including the impacts of climate change, pollution, and anthropogenic activities, all of which contribute to environmental contamination and damage (Alamsyah et al., 2022). Global climate change and local human activities are believed to disrupt the symbiotic relationship between coral polyps and their symbiotic algae, potentially impacting the ecological balance of coral reefs (Lee et al., 2022).

Global climate change negatively affects the mutualistic relationship between corals and their endosymbionts, making coral reefs more susceptible

to bleaching and mass mortality. It also harms Symbiodiniaceae populations in the ocean (Dutra et al., 2021). This event is marked by long-term changes in temperature and precipitation patterns, and involves other elements such as atmospheric pressure and humidity levels in the environment (Abbass et al., 2022). According to the IPCC (2023), in the last two decades (2001–2020), global surface temperatures were recorded 0.99 (0.84 to 1.10) °C higher compared to the period from 1850–1900. The El Niño-Southern Oscillation (ENSO) is the most influential climate phenomenon on an interannual timescale (Tao et al., 2024). Southeast Asia is one of the regions most vulnerable to climate change (Collins et al., 2013; Nguyen-Le, 2024).

Regional-scale coral bleaching is strongly associated with rising temperatures, especially during recurring El Niño–Southern Oscillation (ENSO) events (Hughes et al., 2003). Climate-induced coral bleaching is becoming more frequent across all phases of ENSO. As global warming progresses, tropical sea surface temperatures are now higher during La Niña conditions than they were during El Niño events three decades ago (Hughes et al., 2018). Corals experiencing stress from excessively high temperatures expel most of their

endosymbionts, causing them to become pale or white (Hughes *et al.*, 2003). Coral bleaching is a phenomenon where corals experience symbiotic damage. It is a physiological response to environmental stressors, leading to the expulsion of symbiotic algae that provide color to the corals, leaving the coral structure white (Janzen *et al.*, 2021). Bleached corals often experience reduced growth rates, decreased reproductive capacity, increased susceptibility to disease, and higher mortality rates (Satyaveer *et al.*, 2022). The survival of corals in the ocean is closely linked to their symbiotic algae. According to LaJeunesse *et al.* (2009), coral bleaching can speed up changes in coral populations so that individual corals will choose symbiotic algae that are tolerant of change. Therefore, information on the structure of the Symbiodiniaceae community in corals is important to study to understand how coral symbiosis with symbiotic algae supports coral growth and how the corals are damaged during bleaching.

Karimunjawa is one of marine national parks, which plays a vital role in the conservation of Indonesia's marine ecosystems. It offers enchanting snorkelling and diving spots with coral reefs and biodiversity within. The high number of tourists, along with various anthropogenic activities and natural phenomena, leads to environmental changes that can increase the vulnerability of coral hosts, enhance pathogen virulence, and result in coral disease outbreaks (Sabdono *et al.*, 2019). In addition, increased sea traffic, including merchant ships, tourist ships and fishing vessels crossing the Karimunjawa Marine National Park, also has the potential to disturb and damage coral reefs (Sabdono *et al.*, 2024).

Sambangan Island is one of 27 islands in Karimunjawa. This uninhabited island is known as a marine tourism destination, surrounded by a coral reef ecosystem, and also features floating net cages for fish farming. The development of tourism and fish farming can certainly boost the local economy. However, tourism activities, if not managed properly, can degrade environmental quality. This decline in environmental conditions may lead to damage and even the extinction of marine life. Increased marine pollution creates unstable conditions for the organisms living in these waters. According to Sabdono *et al.*, (2017), human activities have significantly impacted coral reef ecosystems in the Java Sea through pollution and marine aquaculture. The enrichment of nutrients has a negative effect on coral reefs.

Understanding coral-algae symbiosis and how it responds to extreme environmental conditions is crucial for predicting the future of coral reef

ecosystems (Ip *et al.*, 2022). In recent decades, numerous studies have focused on identifying various Symbiodiniaceae species using molecular genetic analysis. Several molecular markers, including the 18S, ITS, and 28S regions of the rDNA gene, have been employed for this purpose (Saad *et al.*, 2020).

Symbiodiniaceae family includes several genetically distinct lineages, ranging from Clade A to Clade J, where each clade is now assigned at genus level (Pochon and Gates, 2010; LaJeunesse *et al.* 2018; Yorifuji *et al.*, 2021). Currently, 15 genera of Symbiodiniaceae have been identified both morphologically and phylogenetically (LaJeunesse *et al.*, 2018). Different species of Symbiodiniaceae have distinct optimal physiological requirements and stress tolerances, leading to varied phenotypic expressions in their coral hosts (Boulette *et al.*, 2016). Some adult coral species can alter their symbiont communities to quickly adapt to changing environmental conditions (Torda *et al.*, 2017). Corals dominated by Symbiodiniaceae *Durudinium* (previously known as Clade D) are generally more tolerant to temperature changes, whereas *Cladocopium* (formerly Clade C) is considered more sensitive (van Oppen, 2009; Quigley *et al.*, 2018b). Research on Symbiodiniaceae in Indonesia are still very limited, particularly regarding corals affected by diseases such as coral bleaching. This study aims to identify the species of Symbiodiniaceae in coral bleaching samples from Sambangan Island, Karimunjawa, using molecular genetic approach.

Materials and Method

Bleaching coral fragments were collected using SCUBA diving from Sambangan Island, Karimunjawa, in February 2024. Samples were collected from shallow waters at the depths of 1–3 meters across three sampling points: ST1 (Reef Patch in front of Sambangan Island), ST2 (South of Sambangan Island), and ST3 (East of Sambangan Island) (Figure 1). 21 samples from eight different coral genera were collected by cutting fragments, 3–5 cm in length. Living branches of coral colonies (both hard and soft corals) that was white or pale in color but still have intact tissue polyp were carefully cut using pliers, referring to Wijayanti *et al.*, (2017). Samples were identified by examining the colony shape, polyp structure, and corallite form. This identification process was carried out by matching the type or characteristics corals with several reference books such as Reef Finder (Kelley, 2015), Soft Corals and Sea Fans: A Comprehensive Guide to the Tropical Shallow Water Genera of the Central-West Pacific, the Indian Ocean and the Red Sea (Fabricius *et al.*, 2001), Reef Finder (Kelley, 2015)

and Corals of The World (Veron, 2000). All collected fragment samples were stored in tubes and preserved with a DNA/RNA Shield solution to ensure their integrity before molecular analysis. The subsequent laboratory analysis was conducted at the Integrated Laboratory of Diponegoro University.

Coral tissue, along with its symbiotic algae, was collected using the extraction and amplification techniques outlined by LaJeunesse (2004). DNA extraction from the tissue was carried out using a Geneaid Genomic DNA Mini Kit (Plant) (Geneaid Biotech Ltd, Taiwan) following the manufacturer's procedures. The extracted samples were then subjected to DNA amplification using the PCR (Polymerase Chain Reaction) method. DNA obtained at the extraction stage was used as a template for the amplification process. The target region used was ITS (Internal Transcribed Spacer). The primers used were r18Sf (5'-GAAAGTTTCATGAACCTTAT-3') and Sym28Sr (5'-CTTGTRTGACTTCATGCTA-3') for the ITS-rDNA region (Yamashita *et al.*, 2013). DNA amplification was carried out by following the procedure by Yamashita *et al.* (2011), with the following cycles: pre-denaturation one cycle at 94°C

(5 min), denaturation 35 cycles at 94°C (45 sec), annealing 51°C (45 sec), extension 72°C (1 min), and post-extension one cycle at 72°C (7 min). Among 21 samples, only 18 were successfully extracted and amplified.

The amplified DNA was then visualized by electrophoresis using gel media. 1% agarose gel media, then added with 2 µl of Fluorescent DNA Stain which functions to produce color when exposed to UV light. The electrophoresis was carried out at 100 Volts, 400 A for 30 minutes and the DNA of the samples were observed with a UV transilluminator. The size of the PCR DNA results was compared with the marker (ladder) to determine the length of the sample DNA. The PCR results of the samples that have been electrophoresed and were in accordance with the target are then sent to PT. Genetika Science Indonesia (Jakarta) for further nucleotide base sequencing or sequencing. Sequencing was carried out using the Sanger method according to the company protocol.

The sequencing data obtained (500 – 750 bp length) were then read using the MEGA 10

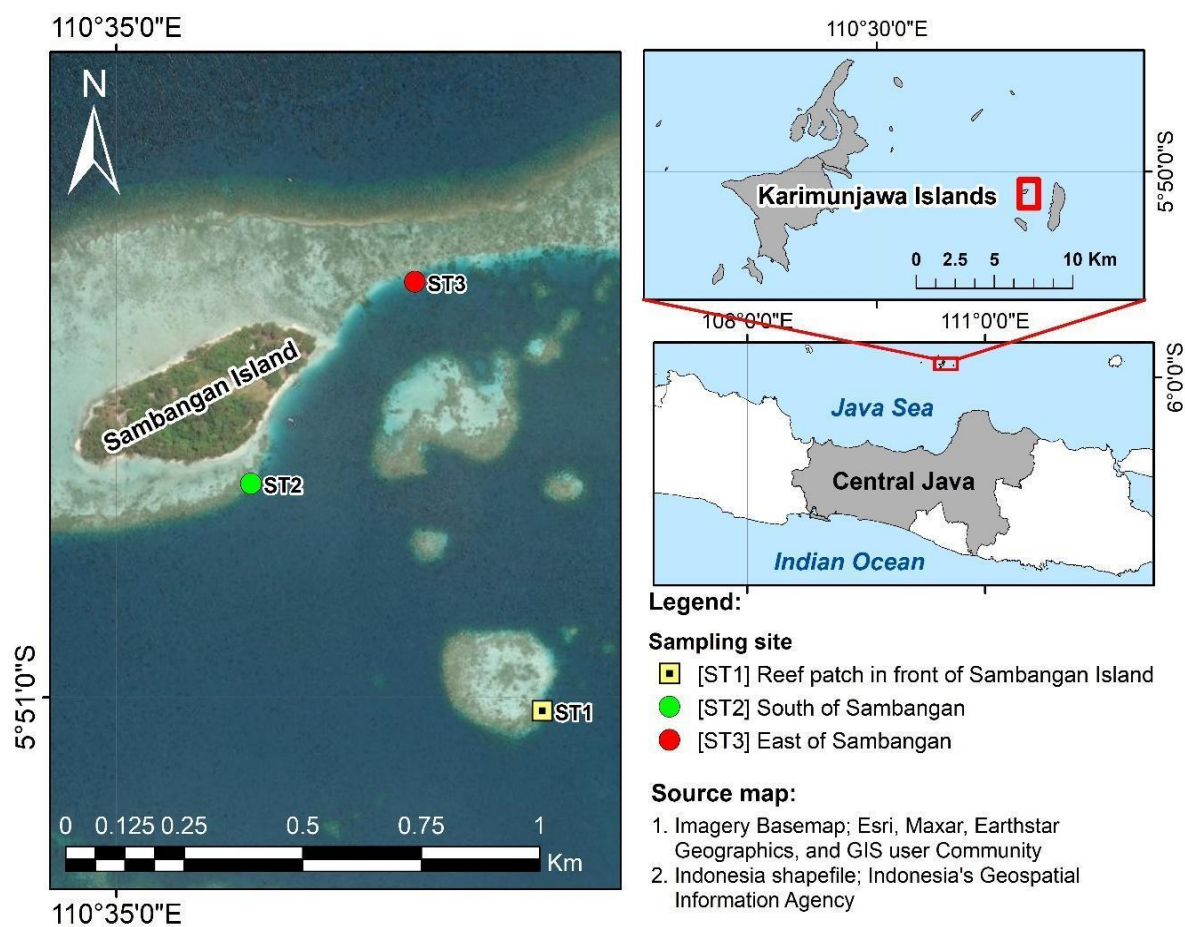


Figure 1. Research Location Map (Sambangan Island, Karimunjawa).

(Molecular Evolutionary Genetic Analysis) application and aligned using ClustalW. Then the alignment data was matched/identified in the gene database BLAST (The Basic Local Alignment Search tool) from NCBI (National Center for Biotechnology Information) (<http://blast.ncbi.nlm.nih.gov/>) to ensure that the sample was in accordance with the research target. Phylogenetic analysis was made to see the kinship between species/genus from the 18 samples. The creation of a phylogenetic tree was carried out using the MEGA 10 software, the Maximum likelihood method with Kimura-2 model and a bootstrap replication of 1000. Genetic distance was calculated to determine the relationship between species/genus from statistical calculations interpreted in the form of numbers, calculations were carried out using the 2-parameter Kimura model (Loh et al, 2001).

Results and Discussion

The coral bleaching samples were from the genera *Acropora*, *Montipora*, *Pocillopora*, *Echinopora*, *Pectinia*, *Heliopora*, *Lobophytum*, and *Sarcophyton* (Figure 2). The BLAST analysis shows that the Symbiodiniaceae in the bleaching coral samples were closely related to *Cladocopium goreau*, *Cladocopium* sp., and *Symbiodinium* sp. (now emended as *Cladocopium* sp.), all of which

belong to the genus *Cladocopium* (Figure 3) with a similarity percentage ranging from 97% to 100%. (Table 1).

The genetic diversity of these coral endosymbionts reflects their varying stress tolerance, influenced by differences in photosynthetic efficiency and environmental adaptability among Symbiodiniaceae genotypes (Russnak et al., 2021). The genus *Cladocopium* is highly adaptable and forms symbiotic relationships with a wide range of hosts, including corals and other cnidarians, such as clams, ciliates, flatworms, foraminifera, and sponges (LaJeunesse et al., 2018), although one experimental research suggested that a *Cladocopium* strain did not compatible with a species of *Cassiopea* (Hudatwi et al., 2020).

The genus *Cladocopium*, identified in this study, is known for its diverse physiological traits that enhance the disease resistance of its hosts (Wijayanti et al., 2022). This genus is widely distributed within the Symbiodiniaceae family and forms symbiotic relationships with numerous coral species (Huang et al., 2024). In coral colonies, Symbiodiniaceae are often regarded as indicators of tolerance to environmental stress (Beltran et al., 2021). *Cladocopium* is commonly found in the Indo-

Table 1. Results of homology analysis of Symbiodiniaceae diversity from Sambangan Island, Karimunjawa.

No	Sample Code	BLAST result	Base pair	Query Cover (%)	Percent Identity (%)	Accession Code of Closest Taxa	Accession Code of Sample
1.	CB1	<i>Cladocopium goreau</i>	650	100	100	ON705574.1	PQ632543
2.	CB2	<i>Cladocopium goreau</i>	659	100	100	ON705623.1	PQ632544
3.	CB3	<i>Cladocopium goreau</i>	655	100	100	ON705616.1	PQ632545
4.	CB4	<i>Cladocopium</i> sp.	589	99	98.30	MN654291.1	PQ632546
5.	CB5	-	-	-	-	-	-
6.	CB6	-	-	-	-	-	-
7.	CB7	<i>Cladocopium goreau</i>	620	100	99.35	ON705587.1	PQ632547
8.	CB8	<i>Cladocopium goreau</i>	654	100	99.85	ON705596.1	PQ632548
9.	CB9	<i>Cladocopium goreau</i>	670	99	100	ON705624.1	PQ632549
10.	CB10	<i>Cladocopium goreau</i>	595	100	99.00	ON705587.1	PQ632550
11.	CB11	<i>Symbiodinium</i> sp.	652	100	99.69	AB665632.1	PQ632551
12.	CB12	<i>Symbiodinium</i> sp.	612	100	98.86	EU786050.1	PQ632552
13.	CB13	<i>Symbiodinium</i> sp.	602	99	98.67	AB665617.1	PQ632553
14.	CB14	<i>Symbiodinium</i> sp.	540	100	99.63	AB665645.1	PQ632554
15.	CB15	<i>Symbiodinium</i> sp.	655	100	99.69	EU828669.1	PQ632555
16.	CB16	-	-	-	-	-	-
17.	CB17	<i>Cladocopium goreau</i>	654	100	100	ON705623.1	PQ632556
18.	CB18	<i>Cladocopium goreau</i>	632	99	98.89	ON705584.1	PQ632557
19.	CB19	<i>Symbiodinium</i> sp.	588	99	98.30	LC368864.1	PQ632558
20.	CB20	<i>Cladocopium goreau</i>	682	99	99.56	ON705588.1	PQ632559
21.	CB21	<i>Symbiodinium</i> sp.	584	99	99.83	AB665632.1	PQ632560

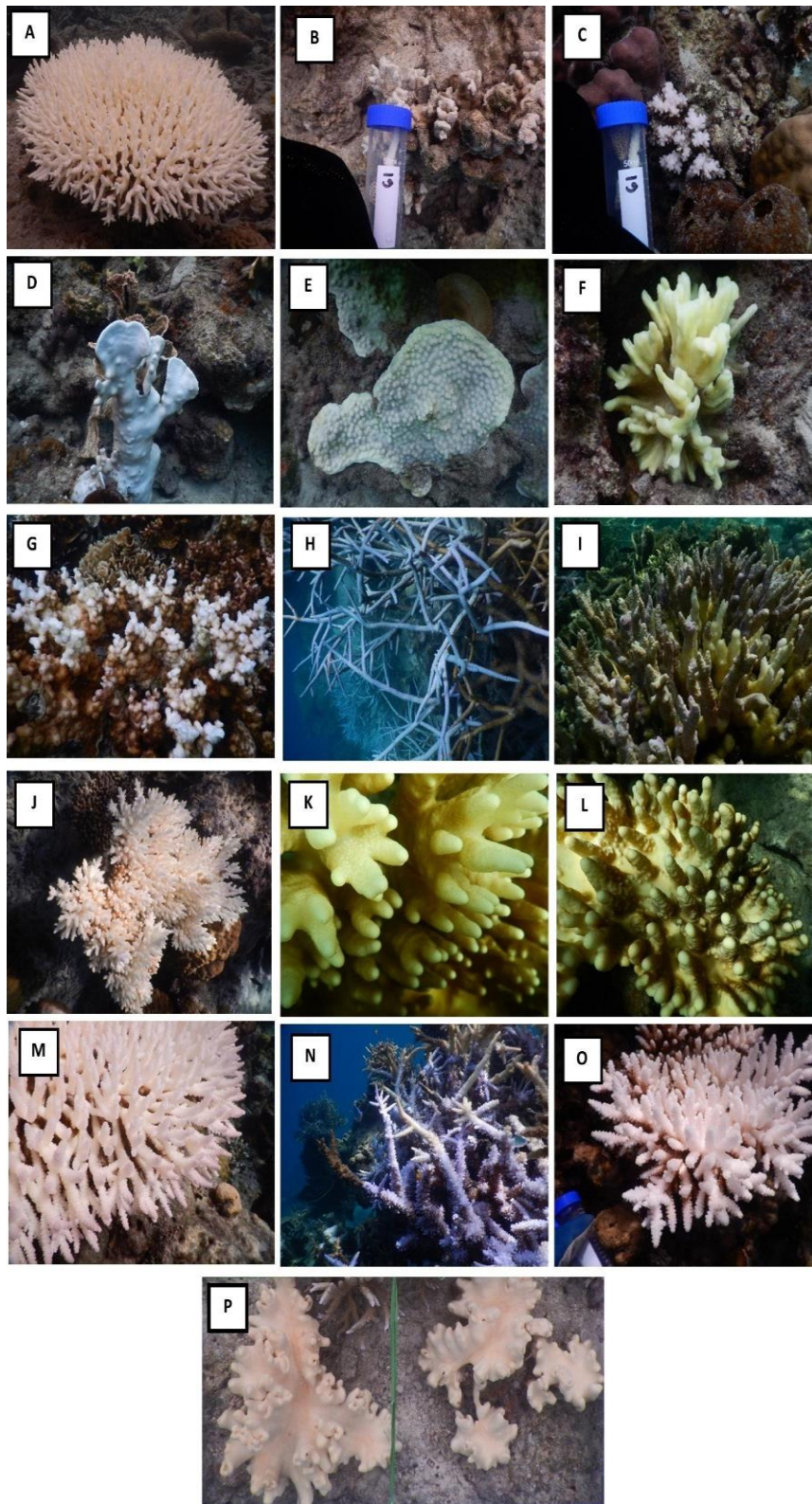


Figure 2. Coral Bleaching Sample Colony : (A) *Acropora* (Arborescent Tabulate) (CB1); (B) *Montipora* (Branching) (CB2); (C) *Pocillopora* (Submassive) (CB3); (D) *Heliopora* (Laminar) (CB4); (E) *Echinopora* (laminar) (CB5); (F) *Pectinia* (Branching) (CB6); (G) *Echinopora* (branching) (CB7); (H) *Acropora* (Branching) (CB8); (I) *Montipora* (Branching) (CB9); (J) *Acropora* (Branching) (CB10); (K) Soft coral *Sclerophyton* (Digitate) (CB11); (L) *Lobophytum* (Digitate) (CB12); (M) *Acropora* (Tabulate) (CB13); (N) *Acropora* (Branching) (CB14); (O) *Acropora* (Arborescent Tabulate) (CB15); (P) Soft coral *Sarcophyton* (CB16).

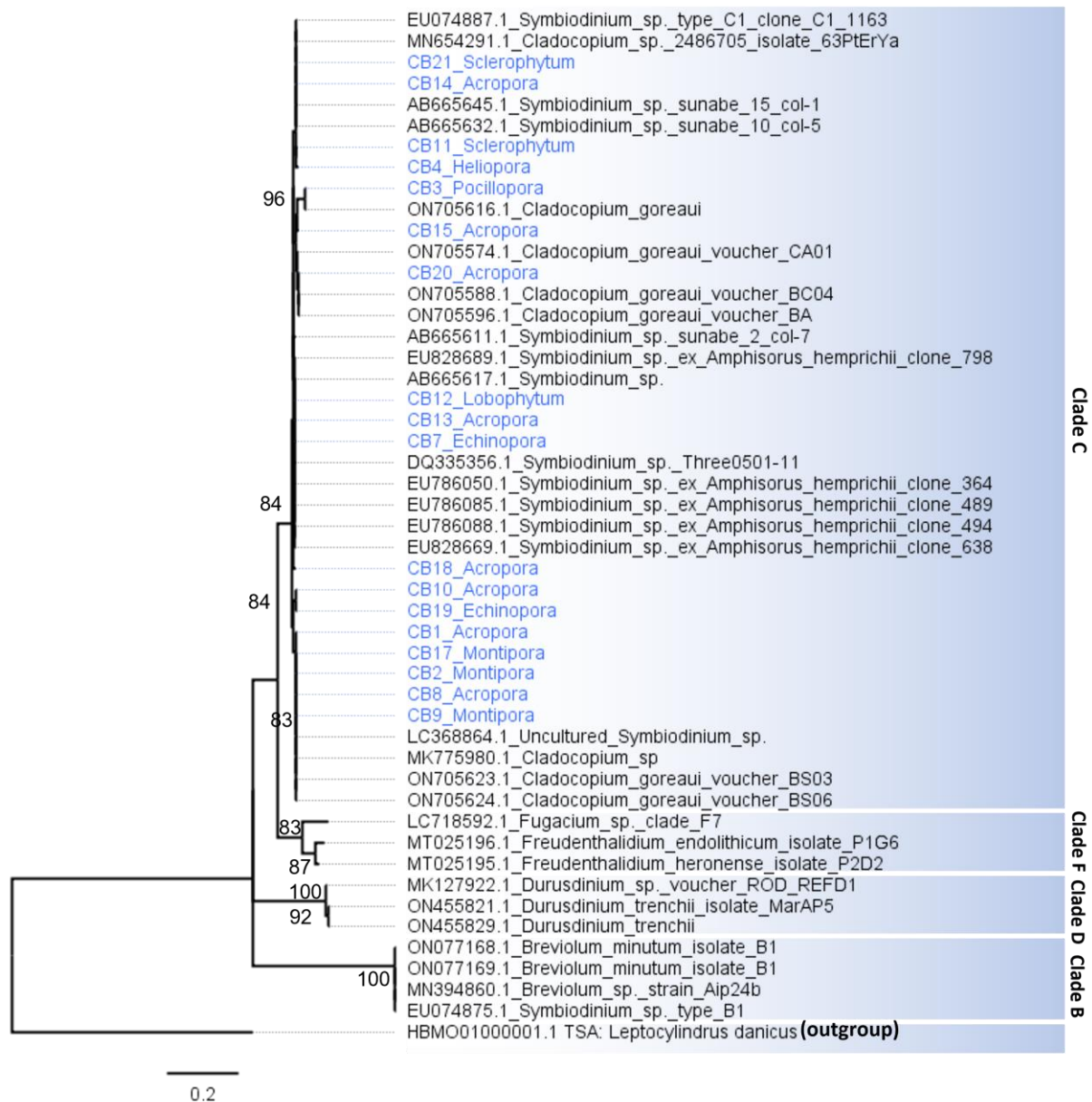


Figure 3. Phylogenetic tree of symbiotic algae from coral bleaching samples of Sambangan Island, Karimunjawa Archipelago, constructed using Maximum Likelihood method and Kimura2 model. Samples from this research are presented in blue. 'CB' in sample code refers to coral bleaching, followed by sample number and host genus. Ingroups and outgroups were written with accession code followed by species name and source (when applicable).

Pacific region alongside the genus *Durusdinium* (Lajeunesse *et al.*, 2018; Leveque *et al.*, 2019), with its dominant species being *C. goreau* (previously type C1) (Lajeunesse, 2004; Bongaerts *et al.*, 2015; Chen *et al.*, 2022). This study is also consistent with previous reports that *Cladocopium* is predominantly found in Indonesian waters (Wijayanti *et al.*, 2022; Wijayanti *et al.*, 2025). The composition of Symbiodiniaceae communities associated with hosts can fluctuate over time in response to

environmental changes or during different stages of the host's life history (Poland and Coffroth, 2017).

Some corals exhibit flexibility in their endosymbiont communities, such as shifting from the dominant genus *Cladocopium* to *Durusdinium* during bleaching events (Ip *et al.*, 2022). This adjustment can occur through two mechanisms: acquiring new Symbiodiniaceae from the surrounding environment ("switching")

Table 2. Genetic Distance of the sample (Pairwise distance : Kimura-2 parameter)

	CB1	CB2	CB3	CB4	CB7	CB8	CB9	CB10	CB11	CB12	CB13	CB14	CB15	CB17	CB18	CB19	CB20	CB21
CB1																		
CB2	0.094																	
CB3	0.124	0.036																
CB4	0.129	0.040	0.023															
CB7	0.116	0.029	0.011	0.019														
CB8	0.000	0.094	0.124	0.129	0.116													
CB9	0.094	0.000	0.036	0.040	0.029	0.094												
CB10	0.111	0.025	0.019	0.023	0.008	0.111	0.025											
CB11	0.122	0.034	0.013	0.019	0.009	0.122	0.034	0.017										
CB12	0.116	0.029	0.015	0.019	0.008	0.116	0.029	0.011	0.013									
CB13	0.118	0.030	0.017	0.021	0.009	0.118	0.030	0.013	0.015	0.006								
CB14	0.120	0.032	0.011	0.019	0.007	0.120	0.032	0.015	0.002	0.011	0.013							
CB15	0.118	0.030	0.008	0.021	0.006	0.118	0.030	0.013	0.011	0.009	0.011	0.009						
CB17	0.094	0.000	0.036	0.040	0.029	0.094	0.000	0.025	0.034	0.029	0.030	0.032	0.030					
CB18	0.118	0.030	0.013	0.021	0.002	0.118	0.030	0.009	0.011	0.009	0.011	0.009	0.008	0.030				
CB19	0.105	0.013	0.030	0.034	0.023	0.105	0.013	0.015	0.028	0.023	0.025	0.027	0.025	0.013	0.025			
CB20	0.120	0.032	0.013	0.023	0.007	0.120	0.032	0.015	0.009	0.011	0.013	0.008	0.006	0.032	0.009	0.027		
CB21	0.118	0.030	0.013	0.017	0.009	0.118	0.030	0.013	0.004	0.009	0.011	0.002	0.011	0.030	0.011	0.025	0.009	

or altering the relative abundance of Symbiodiniaceae already present within the coral ("shuffling") (Baker, 2003; Morgans *et al.*, 2020). While not all *Cladocopium* species are temperature tolerant, certain types can provide significant protection corals by reducing bleaching risk (Varasteh *et al.*, 2017; Varasteh *et al.*, 2024).

The phylogenetic tree topology, constructed using the Maximum Likelihood clustering method with bootstrapping, is shown in Figure 3. All samples formed a single clade, with genetic distance values ranging from 0 to 0.129 (Table 2). According to the genetic distance categories defined by Nei (1972) and Wijayanti *et al.* (2022), the low range is 0.01–0.09, the medium range is 0.1–0.9, and the high range is 1.0–2.0. In this study, all samples fell within the low to medium range, indicating a close phylogenetic relationship. The maximum genetic distance observed in this study is 0.129, although this value is slightly higher than the upper limit of the low category, it is still within the beginning of the medium category. This indicates that there is a moderate level of genetic difference among the samples. However, the difference is not large enough to suggest major evolutionary separation or that the samples belong to completely different species. This kind of genetic variation might be caused by factors like adaptation to local environments, small ongoing genetic changes (microevolution), or the separation of populations within species that lives across a wide area. Complex evolutionary process including intragenomic variation and concerted evolution can further influence interpretations of genetic distance and phylogenetic relationships (LaJeunesse *et al.*, 2018). An outgroup was included for comparison, confirming that the 11 samples were not from the same genus as the outgroup.

Coral identification results indicate that all samples from various hard coral classes (*Acropora*, *Montipora*, *Pocillopora*, *Heliopora*, *Echinopora*, *Pectinia*) and soft coral (*Lobophytum* and *Sclerophytum*) were found to be in symbiosis with the *Cladocopium* genus of Symbiodiniaceae. This suggests that the *Cladocopium* genus is likely widely distributed in the waters of the Karimunjawa Islands.

However, since sampling in this study was not conducted periodically and only occurred during coral bleaching events, the type of Symbiodiniaceae present before bleaching remains unknown. Several possibilities include: 1) Prior to bleaching, corals may have hosted multiple Symbiodiniaceae genera, however, following bleaching, only certain members of the *Cladocopium* genus persisted. 2) Alternatively, the *Cladocopium* genus may have dominated the coral symbiosis from the beginning and continued to remain after the bleaching event.

While all Symbiodiniaceae in the samples belong to one genus *Cladocopium*, ten of them are closely related to *C. goreau*. This species, is known for its high physiological diversity and broad distribution. *C. goreau* maintains a mutualistic relationship with various coral hosts, playing a critical role in helping corals adapt to varying light intensities and temperatures (LaJeunesse *et al.*, 2018; Zhang *et al.*, 2023). According to Morgans *et al.* (2020), *C. goreau* is a temperature-tolerant Symbiodiniaceae species that significantly reduces coral mortality during bleaching events by enhancing photosynthetic efficiency.

Conclusion

Research on the genetic diversity of Symbiodiniaceae in relation to coral bleaching can help clarify the role of symbiont genotypes in coral resilience. In this study, all bleached coral samples—both hard and soft corals—from Sambangan Island, Karimunjawa, were found to host symbionts from the *Cladocopium* genus, particularly *C. goreau*. This symbiont species is known for its tolerance to temperature fluctuations, which supports the survival and fitness of its coral hosts, especially during bleaching events.

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