

Short communication: Algal genotypes in White Syndrome infected coral *Acropora muricata* from the Karimunjawa Islands, Indonesia

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Abstract. Wijayanti DP, Nadia M, Indrayanti E, Haryanti D, Bachtiar M. 2021. Short communication: Algal genotypes in White Syndrome infected coral *Acropora muricata* from the Karimunjawa Islands, Indonesia. *Biodiversitas* 23: 415-423. The physiological equilibrium of the coral holobiont may help the corals to survive under various environmental threats. The symbiont genotype is thought to play an important role in the host's susceptibility to thermal stress and coral disease incidence. We identified the Symbiodiniaceae diversity of coral *Acropora muricata* infected by White Syndrome (WS). Samples were collected from Cemara Kecil Island and Barakuda Beach, Kemujan Island, Karimunjawa Islands. We applied DNA barcoding of 28S nuclear ribosomal RNA gene of the healthy colonies, healthy tissue, and lesions of diseased colonies to perform their symbiont type identification. There were two different symbiont types observed from the phylogenetic analysis, which formed two clades. A clade consisting all Cemara Kecil Island samples while the other with all Barakuda Beach samples except for one. All samples harbored symbiont types from the genus *Cladocopium*. The members of the genus were reported to have various physiological properties which may support the host resilience when facing the disease. A robust study is needed to reveal the influence of symbiont genotypes on the occurrence of coral disease and the susceptibility of the coral host.

Keywords: *Acropora muricata*, coral disease, DNA barcoding, white syndrome

INTRODUCTION

During the Anthropocene, coral reefs have experienced severe degradation due to global climate change, pollution, and other human-made disturbance. The shorter interval between recurrent bleaching episodes inhibited the full recovery of coral assemblages (Hughes et al. 2018) and disease outbreaks (Weil and Rogers 2011; Shore-Maggio et al. 2018) have a detrimental effect on the coral reef ecosystem.

The reef-building corals have formed mutually beneficial symbioses with unicellular photosynthetic algae, commonly known as zooxanthellae, a member of the family Symbiodiniaceae. The symbiotic algae translocate up to 95% photosynthetic fixed carbon to the coral host daily for their metabolic needs (Muscatine et al. 1984). There are numerous studies regarding the coral and Symbiodiniaceae interaction (Stambler 2011; Hidaka 2016) and the impact of climate change on the coral reef ecosystems (Heron et al. 2016; Szabó et al. 2020). Meanwhile, the impact of epizootics on reef communities around the globe was more discerning such as the Caribbean (Alvarez-Filip et al. 2009), Indo-Pacific (Weil et al. 2012), Great Barrier Reef (Haapkylä et al. 2013), and Hawaii (Aeby et al. 2011). The identification of coral disease causative agents also heavily accelerated (Rosenberg and Kushmaro 2011). However, the primary

causative agent, which fulfills Koch's Postulate, only occurred in a few cases and more diagnostic tools are needed (Peters 2015).

Family Symbiodiniaceae includes several genetically divergent lineages called clades. Clades A through J were reported up to the present (Pochon and Gates 2010; Nitschke et al. 2020; Yorifuji et al. 2021). Recently, some of the clades and subclades were designated as genera. Each clade or genus consists of numerous types. Some of the types were further described as species (LaJeunesse and Thornhill 2011; LaJeunesse et al. 2018).

Studies suggested that functional differences between algal symbionts as well as their genotypes are important factors in coral colonies distribution (Iglesias-Prieto et al. 2004), coral growth (Little et al. 2004; Jones and Berkelmans 2010), symbiont composition and photo-physiology ability (McGinley et al. 2012), and the coral susceptibility to thermal stress (Cunning and Baker 2013; Wham et al. 2017; Manzello et al. 2019). Most corals harbor *Cladocopium* (formally Clade C) (LaJeunesse et al. 2018), which is highly prevalent and ecologically widespread in nearly all reef ecosystems (LaJeunesse et al. 2010; Ziegler et al. 2017). In some cases, the coral host harbors two or more symbiont types simultaneously (LaJeunesse et al. 2008). *Cladocopium* also was reported as the most widely distributed symbiont among coral hosts in

the tropical area (LaJeunesse et al. 2010; Ziegler et al. 2017), with *Symbiodinium thermophilum* presumably the most heat-tolerant member (Hume et al. 2015).

White Syndrome (WS) disease is defined as tissue loss revealing a bare skeleton. Lesion appears from the peripheral, central or basal area of the coral colonies and spread to form a band across the entire colony with moderate to rapid progression. The tissue loss diseases have a similar macroscopic appearance of a white syndrome in the Caribbean, therefore, the term of WS then was applied for similar symptoms on Indo-Pacific corals (Raymundo et al. 2008). *Acropora* is one of the most affected genera, thus, sometimes, the disease was referred to as Acroporid White Syndrome (Roff et al. 2011; Bourne et al. 2014). The disease has now been reported from various reefs throughout the Indo-Pacific (Roff et al. 2008; Luna et al. 2010; Weil et al. 2012). More recently, the Acroporid white syndrome appeared at Palk Bay Reef, southeast coast of India and killed 8% of Acroporid corals (Thinesh et al. 2017). While in Indonesia, the disease was divulged from Wakatobi (Haapkylä et al. 2007) and Kessilampe waters, Southeast Sulawesi (Palupi et al. 2018), Seribu Islands (Johan et al. 2015; Rosyid and Lutfi 2019), Karimunjawa Islands (Sabdono et al. 2019), and Sempu Beach, East Java (Rosdianto et al. 2020).

Karimunjawa Islands, located 80 km north of Jepara, Central Java, represent one of the oldest national marine parks in Indonesia (Campbell et al. 2013). The park consists of 27 islands that are dominated by coral reef ecosystems, with *Acropora* being the most dominant genus (Edinger et al. 2000). Various disease types were observed at the surveyed island around the Archipelago, namely Black Band Disease (Sabdono et al. 2017; Wijayanti et al. 2018), White Syndrome (Sabdono et al. 2019), and White Patch (Wijayanti et al. 2020). During monitoring of the coral disease, we found two different types of diseases infected the coral *Acropora muricata*. The first type appeared like a White Patch Disease that infected the coral *A. muricata* from Barakuda beach, Kemujan Island. The lesions were presented randomly on the coral tissues and each formed a circular patch with various diameters between 2-5 cm (Patterson et al. 2002; Sutherland et al. 2010; Wijayanti et al. 2020). The second type showed a symptom similar to White Syndrome (Roff et al. 2011; Sabdono et al. 2019). Colonies showing White Syndrome were found at Cemara Kecil Island (Figure 2). There was no observation on the progression rate of the diseases and testing of disease precursor, therefore both symptoms were classified as White Syndrome, a collective term attributed

to white lesions followed by tissue necrosis (Bourne et al. 2014).

Although the dynamic relation between symbiotic algae and the coral host has been studied heavily (Muller-Parker et al. 2015), the interactions among Symbiodiniaceae, corals and other coral holobiont is largely unknown. Most studies are focused on whether Symbiodiniaceae remained photosynthetically competent when the coral host was attacked by a coral disease (Cervino et al. 2004; Ulstrup et al. 2007; Roff et al. 2008; Burns et al. 2013). On a side note, symbiont types may also play an important role in the incidence of coral disease and the host fitness (Toller et al. 2001; Stat et al. 2008; Correa et al. 2009; Rouzé et al. 2016). Currently, very limited studies on Symbiodiniaceae genotypes are available from Indonesian reefs (deBoer et al. 2012; Roriris et al. 2017), moreover its relationship with coral disease incidence. Here, we investigated the symbiont of *A. muricata* collected from Karimunjawa Islands, which are attacked by WS disease, to observe whether diseased and apparently healthy coral colonies harbor the same clade of the symbiont. Therefore, the coral susceptibility against coral disease can be understood.

MATERIALS AND METHODS

Study area and sample collection

Samples were collected in March 2020 using scuba diving and applying the time swim method, a reef observation method involving divers for a set of time or distance (Raymundo et al. 2008) at depths of 2 to 4 m of Cemara Kecil Island and Barakuda Beach, Kemujan Island waters (Figure 1). All samples were collected under permission of the Karimunjawa Conservation Office (Balai Taman Nasional Karimunjawa) No. 1392/T.34/TU/SIMAKSI/03/2020. Healthy and diseased *A. muricata* showing White Syndrome symptoms were collected from the study area (Figure 2). In total, 20 coral fragments were obtained from the coral colonies by cutting 3-5 cm long branches using pliers. The coral was identified in situ based on a description by Wallace (1999). Three different types of coral fragments were taken from both locations, namely fragments from healthy colonies and fragments from diseased colonies, which were divided into disease-affected area fragments and healthy area fragments (Table 1). All samples were then stored in a plastic zip and brought ashore for preservation in 95% ethanol before further molecular analysis.

Table 1. Sampling location and number of samples obtained from healthy and diseased coral *Acropora Muricata*

Sampling location	Status of samples	Number
Cemara Kecil Island	(CA) Healthy area from healthy colonies	3
	(CB) Infected area from diseased colonies	3
	(CC) Healthy area from diseased colonies	4
Barakuda Beach, Kemujan Island	(BA) Healthy area from healthy colonies	3
	(BB) Infected fragments from diseased colonies	3
	(BC) Healthy fragments from diseased colonies	4

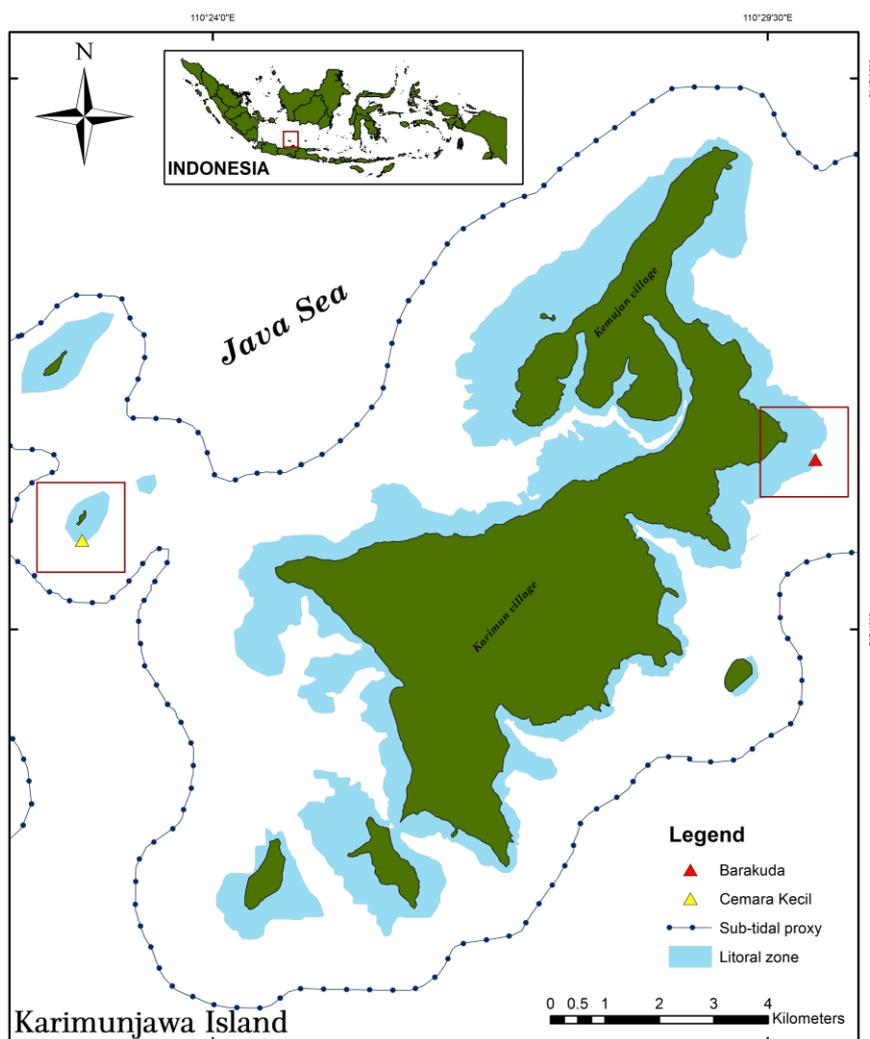


Figure 1. Sampling location at the Cemara Kecil Island (05°50'06.8" S 110°22'42.4" E) and Barakuda beach (05°49'18.5" S 110°29'58.5" E), Kemujan Island, Karimunjawa Islands. Yellow and red triangle represent the sampling locations

DNA extraction, amplification and sequencing

Coral tissue, including symbiotic algae, was obtained following the extraction and amplification method by LaJeunesse et al. (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. Partial sequences of the 28S nuclear ribosomal RNA gene were amplified for all extracted DNA. Primer pair 28S-forward/28S-reverse (Loh et al. 2001) was used for PCR (Polymerase Chain Reaction) and thermal cycling conditions were following primer designers. All PCR products were then analyzed by electrophoresis in 2% agarose gel (FMC Bioproduct, Rockland, ME, USA) in 1x TAE buffer. The gel was then visualized under UV Transilluminator (Uvitec Fire Reader, Cambridge, UK) after being stained with ethidium bromide.

All amplified samples were then sent to PT. Genetika Science (Jakarta, Indonesia) for sequencing. Prior to the sequence procedures, all samples were purified by the company using the gel extraction method. Sequences were

conducted using ABI 3730 × 1 DNA Analyzer (Thermo Fisher Scientific, Massachusetts, USA) according to the company protocols.

Construction of phylogenetic-tree and data analysis

The Basic Local Alignment Search Tool (BLAST) of the NCBI (National Centre for Biotechnology Information) (<http://blast.ncbi.nlm.nih.gov/>), National Institute for Health USA searching was utilized to verify the sequence obtained against the gene database. Alignments of the DNA sequence analysis were performed using CLUSTAL W (Thompson et al. 1994). The phylogenetic tree was constructed using the Maximum likelihood method with a 2-parameter Kimura model as well as the genetic distance. Bootstrap method with 1000 repetitions was applied when constructing the genetic distance (Loh et al. 2001). This analysis was conducted to determine the relationship of an organism from statistical calculations interpreted in the form of numbers. Analysis was performed using MEGA 10.0 software (Magalon et al. 2007).

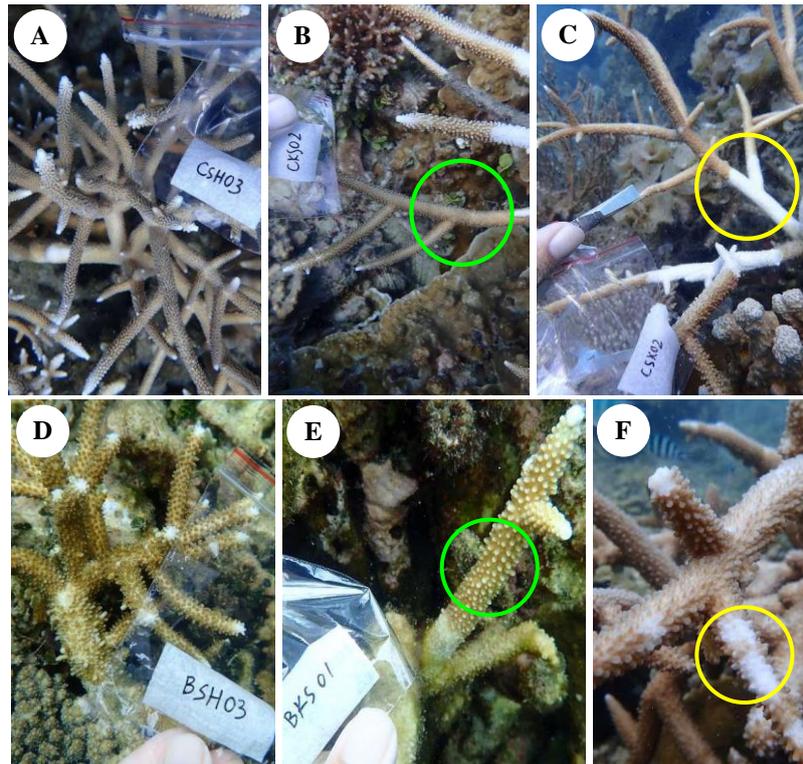


Figure 2. *Acropora muricata* collected from Cemara Kecil Island (A-C) and Barakuda Beach, Kemujan Island (D-F), Karimunjawa National Park. A and D, healthy colony; B and E, healthy area of diseased colony; C and F, infected area of diseased colony; green circle represents the healthy area and yellow circle represents the infected area that were collected for the study

RESULTS AND DISCUSSION

Symbiodiniaceae diversity in healthy and diseased coral *A. muricata*

All 20 samples obtained from the two study sites were successfully extracted and amplified. The length of the amplified 28S rDNA was approximately 600 bp. The homology analysis using BLAST searching of 28S rDNA sequences showed that all samples were matched to previously known *Cladocopium* Symbiodiniaceae with similarities ranging between 98.50-100% (Table 2).

Phylogenetic tree and genetic distance

The Symbiodiniaceae sequences obtained were also analyzed by reconstructing the phylogenetic tree to investigate the relationship between Symbiodiniaceae from Cemara Kecil and Barakuda Beach. Several known Symbiodiniaceae types belonging to genera *Cladocopium* were used as the in group, while *Halluxium pauxillum* represents the outgroup (Table 3).

The Maximum likelihood analysis of Symbiodiniaceae detected from all *A. muricata* samples formed two clades. All samples from Cemara Kecil were grouped in clade 1. The majority of samples from Barakuda Beach are grouped in clade 2, with one exception, BB04, which is included in clade 1. The healthy and diseased colonies collected from

both sampling locations showed no difference in their symbiont (Figure 3).

Based on Nei's (1972) range of genetic distance (the low category ranged from 0.01 to 0.09; moderate 0.1-0.9 and high 1.0-2.0), all samples have a low genetic distance (0-0.021) (Table 4) which, means that all samples have a close relationship in the phylogenetic tree.

Discussion

All samples of *A. muricata* corals collected from both study sites were found to have symbiotic algae, including coral colonies infected by White Syndrome (WS) disease. The members of the genus *Cladocopium*, as found in this study, were reported to have various physiological properties, which may support the host resilience when facing the disease. Cervino et al. (2004) demonstrated that corals exposed to Yellow Blotch disease did not expel their symbionts, in contrast to corals that were stressed due to extreme temperatures. The presence of Symbiodiniaceae in diseased corals may help the host to survive by providing energy from photosynthesis. Baker et al. (2013) demonstrated that coral acquired more nitrogen (N)-bearing amino acids as important photosynthetic resources besides carbon-rich carbohydrates from clade C Symbiodiniaceae, making it a competitive symbiont that dominates coral populations.

Table 2. Homology analyses of Symbiodiniaceae diversity of *Acropora muricata* from the sampling locations; CC: Cemara Kecil healthy area of diseased colony; CB: Cemara Kecil infected area of diseased colony; CA: Cemara Kecil healthy colony; BC: Barakuda Beach healthy area of diseased colony; BB: Barakuda Beach infected area of diseased colony; BA: Barakuda Beach healthy colony

Samples code	Host status	Base pair	BLAST Identification	Query Cover (%)	Identify (%)	Accession Code
CC01	White Syndrome	603	<i>Symbiodinium thermophilum</i>	99	98.50	KR996308.1
CC02	White Syndrome	583	<i>Symbiodinium</i> sp. clade C	100	98.80	GQ984275.1
CC03	White Syndrome	598	<i>Symbiodinium thermophilum</i>	100	98.66	KR996308.1
CB01	White Syndrome	598	<i>Symbiodinium thermophilum</i>	100	98.66	KR996308.1
CB02	White Syndrome	600	<i>Symbiodinium thermophilum</i>	100	98.67	KR996308.1
CB03	White Syndrome	573	<i>Symbiodinium</i> sp. C3	100	98.78	KF740676.1
CB04	White Syndrome	600	<i>Symbiodinium thermophilum</i>	100	98.67	KR996308.1
CA01	Healthy	573	<i>Symbiodinium</i> sp. C3	100	98.78	KF740676.1
CA02	Healthy	598	<i>Symbiodinium thermophilum</i>	100	98.66	KR996308.1
CA03	Healthy	593	<i>Symbiodinium</i> sp. clade C	100	98.65	GQ984275.1
BC01	White Syndrome	580	<i>Symbiodinium</i> sp. clade C	100	100	GQ984257.1
BC02	White Syndrome	582	<i>Symbiodinium</i> sp. clade C	100	100	GQ984257.1
BC03	White Syndrome	583	<i>Symbiodinium</i> sp. clade C	100	99.83	GQ984257.1
BB01	White Syndrome	551	<i>Symbiodinium</i> sp. C40	99	100	KF740681.1
BB02	White Syndrome	594	<i>Symbiodinium</i> sp. clade C	100	99.33	GQ984257.1
BB03	White Syndrome	602	<i>Symbiodinium</i> sp. clade C	100	99.83	GQ984257.1
BB04	White Syndrome	602	<i>Symbiodinium thermophilum</i>	100	98.67	KR996308.1
BA01	Healthy	579	<i>Symbiodinium</i> sp. clade C	100	99.83	GQ984257.1
BA02	Healthy	592	<i>Symbiodinium</i> sp. clade C	100	99.83	GQ984257.1
BA03	Healthy	603	<i>Symbiodinium</i> sp. clade C	100	99.66	GQ984257.1

Table 3. Outgroup and Ingroup samples data from the GenBank Database

Accession Code	Host	Base pair	BLAST Identification (named after La Jeunesse et al. 2018)	Location
KR996308	<i>Porites lutea</i>	823	<i>Cladocopium thermophilum</i> (previously <i>Symbiodinium thermophilum</i>)	United Arab Emirates: Persian/Arabian Gulf
MK696600	-	600	<i>Cladocopium goreau</i>	-
MT022024	-	612	<i>Halluxium pauxillum</i>	-

Ulstrup et al. (2007) suggested that Symbiodiniaceae did not lose its competence for photosynthesis when the host is affected by the disease. Roff et al. (2008) found that corals with WS lesions and the healthy colonies contain a similar density of Symbiodiniaceae. Moreover, although the infected area of the host tissue was extensively degraded, the algae remain physiologically uncompromised. In contrast, different results were reported from the tissue that was affected by Growth Anomaly where the symbiont in the coral tissue was photochemically compromised compared to those inhabiting the healthy tissue (Burns et al. 2013).

There is no different Symbiodiniaceae clade between the healthy and diseased colonies. All Symbiodiniaceae were identified as members of the genus *Cladocopium*. It was suggested that different symbiont clades might affect the fitness of the coral host. Long-term observation by Rouzé et al. (2016), found that predisposition of disease development in *Acropora cytherea* by *Vibrio* spp depended on the clade harbored by the coral. However, Correa et al. (2009) showed that the diseased corals harbor similar types

of Symbiodiniaceae with the healthy colonies. The corals that were infected with the disease have no 'atypical parasitic' Symbiodiniaceae, a unique type of symbiont which appearances were reported to have a correlation with the incidence of coral disease (Toller et al. 2001).

The BLAST searching showed the healthy and diseased coral colonies contained similar clade of algae both in Cemara Kecil Island and the Barakuda Beach, Kemujan Island. All colonies harbor the genus *Cladocopium* (LaJeunesse et al. 2018). The genus is known to have the most species-rich members within the *Cladocopium* (LaJeunesse et al. 2018), which are found in various types of hosts, in different habitats, and at different depths (Magalon et al. 2007). The members of the genus are broadly distributed (Howells et al. 2020) and ecologically abundant in various coral reef communities in Indo-Pacific (Leveque et al. 2019; Lim et al. 2019). The members of the genus are also reported to have a high thermal tolerance (Howells et al. 2020; Lee et al. 2020). However, Correa et al. (2009) suggested that the diseased colonies has not correlated with the specific *Symbiodinium* types.

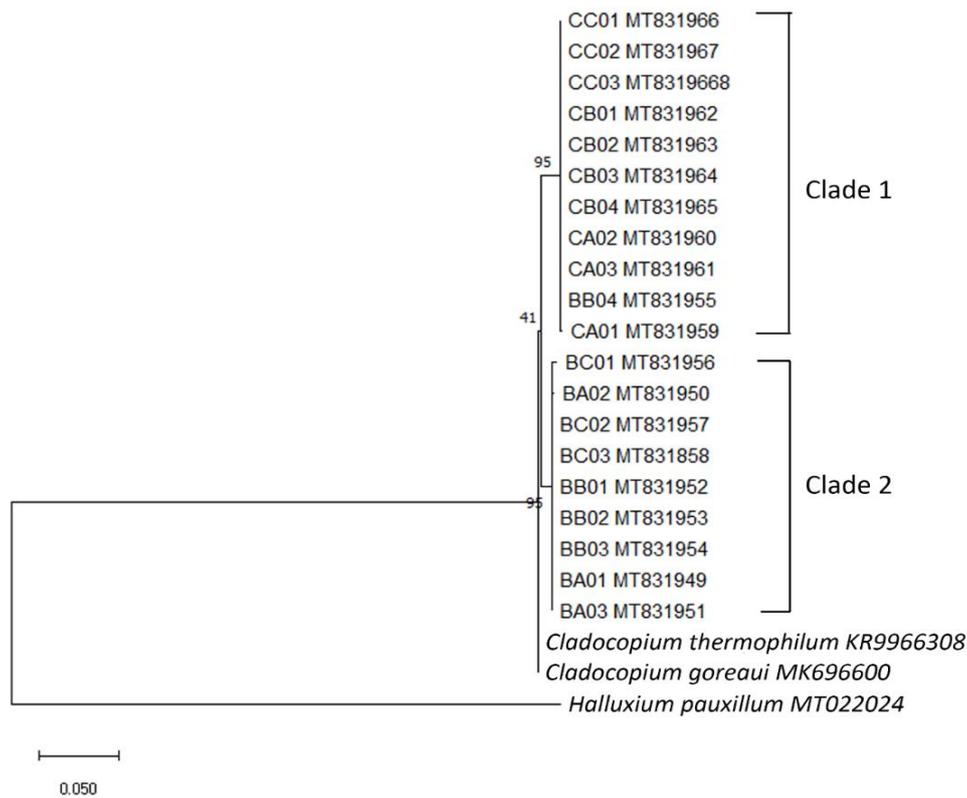


Figure 3. Maximum likelihood tree based on the 28S rDNA sequence data (919 bp after aligned) showing the relationships of zooxanthellae clade with the most closely related zooxanthellae recorded in the GenBank database. The samples obtained from healthy and diseased colonies of *Acropora muricata* were grouped into 2 clades, with the deposited accession number therein (see Table 3 for the outgroup and ingroup samples explanation). CC: Cemara Kecil healthy area of diseased colony; CB: Cemara Kecil infected area of disease colony; CA: Cemara Kecil healthy colony; BC: Barakuda Beach healthy area of diseased colony; BB: Barakuda Beach infected area of diseased colony; BA: Barakuda Beach healthy colony

Table 4. Matrix of Genetic Distance among Samples

	CC01	CC02	CC03	CB01	CB02	CB03	CB04	CA01	CA02	CA03	BC01	BC02	BC03	BB01	BB02	BB03	BB04	BA01	BA02	BA03		
CC01																						
CC02	0																					
CC03	0	0																				
CB01	0	0	0																			
CB02	0	0	0	0																		
CB03	0	0	0	0	0																	
CB04	0	0	0	0	0	0																
CA01	0.002	0.002	0.002	0.002	0.002	0.002	0.002															
CA02	0	0	0	0	0	0	0	0														
CA03	0	0	0	0	0	0	0	0.002	0													
BC01	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.021	0.022	0.022												
BC02	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003											
BC03	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0										
BB01	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0	0									
BB02	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0	0	0								
BB03	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0	0	0	0							
BB04	0	0	0	0	0	0	0	0.002	0	0	0.022	0.019	0.019	0.019	0.019							
BA01	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0	0	0	0	0	0.019					
BA02	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.022	0.021	0.02	0.005	0.002	0.002	0.002	0.002	0.002	0.021	0.002				
BA03	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021	0.019	0.019	0.003	0	0	0	0	0	0.019	0	0.002			

The maximum likelihood clustering analysis showed that the samples were classified into 2 clades, regardless of being obtained from healthy or diseased colonies (Figure 3). The BB04 sample is the only sample collected from Barakuda Beach that is positioned in the first clade. The long-distance dispersal of Symbiodiniaceae may rely on the sea current (Thornhill et al. 2017). The possible explanation as to why the BB04 was found in the first clade is the occurrence of the current movement. Karimunjawa is an archipelago consisting of 27 islands and is located in the Java Sea. Surface currents in the Java Sea are driven primarily by monsoonal winds. Wind and current directions are changed during the east and west monsoons (Edinger et al. 2002). Barakuda Beach was situated in Kemujan Island, along the east side of the main island, Karimunjawa. While the Cemara Kecil Island, positioned on the opposite side of the beach, at the west side of the Karimunjawa Island (Figure 1). Indrayanti et al. (2019) stated that current movement in the Karimunjawa Islands was also influenced by tidal movement, causing a bi-directional movement to east and west-southwest currents.

Examining the symbiont genotypes and its correlation with the onset of coral disease may admit the role of symbiont genotypes. It is important to understand the symbiont genotypes which may contribute the fitness of the host during disease incidence. Our study showed that diseased corals and lesion tissue impacted by WS also harbor the same competitive symbiont types as healthy colonies, thus helping coral hosts to maintain fitness under disadvantaged conditions such as WS disease. However, future studies remain to be done to understand the influence of genotype symbionts on the incidence of disease and the susceptibility of coral hosts.

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