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Symbiont Types of Scleractinian Corals in Java and Bali Waters, Indonesia

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Abstract

Several effects of global warming, particularly coral bleaching, have threatened the symbiotic system of coral reefs. Coral reefs perform a reciprocal system that coincides with coral and their algal symbiont, the environment, and human activities. Nowadays, the system has been threatened by local and global stressors, including global warming. Understanding the ecological resilience of coral reefs to temperature stress relied heavily on the functional variations between algae symbionts as well as their genotypes. Indonesian coral reefs which play a critical value in supporting the reef fisheries, have been reported to experience bleaching events. However, a few reports were on the symbiont type of Indonesian corals. This study investigates the Symbiodiniaceae communities of coral hosts of Panjang Island and Karimunjawa Archipelago in Java Sea, and Tulamben Waters, Bali using internal transcribed spacer two (ITS2) amplicon sequencing. In total, 63 sequences out of 119 coral colonies from 16 genera were obtained from Panjang Island, Karimunjawa Archipelago, and Tulamben waters, Bali. All sequences showed approximately 600 bp in the length of the amplified ITS-rDNA region. The BLAST homology analysis of ITS-rDNA region sequences showed that samples matched Cladocopium sp. and Durusdinium sp. with similarities ranging between 96.79-100%. Cladocopium dominated the symbiont type of the Acropora, and Porites, While Durusdinium is the dominant symbiont in the Pavona, Both algal can be found in Pocillopora sp, Montipora sp, Goniastrea sp., and Lobophyllia sp. The occurrence of the two symbiont types suggests that coral species may adopt heat-tolerant Symbiodiniaceae to adapt to the high environmental temperatures.

Keywords: *Cladocopium*, *Durusdinium*, Indonesian waters, Scleractinian, Symbiodiniaceae,

Introduction

Climate change has now become a global issue with a new definition of environmental, economic, and social impact. Some evidence showed that biological systems throughout the earth have also undergone changes (Walther *et al.,* 2002) such as habitat degradation and loss, followed by declining of biodiversity and productivity which lead to taxonomic shifts (Westoby *et al.,* 2022). The coral reefs which comprise a symbiotic system that coexists among various taxon known as coral's holobiont (Stat *et al.,* 2012) are one of the most vulnerable ecosystems under climate change.

The symbiotic system of coral reefs has been threatened by various factors of global warming in the last several decades (Kayanne, 2016), especially coral bleaching. The recurrent bleaching frequency and the increased severity of coral bleaching events inhibited the full recovery of coral assemblages, indicating that the coral reef ecosystem is fast approaching a critical survival threshold (Hoegh-Guldberg *et al.,* 2017). Since the worldwide bleaching event in 1997-1998 (Wilkinson *et al.,* 1999), coral reefs have experienced another two global mass bleaching events, in 2010 and 2015-2017 (Eakin *et al.,* 2019). During the last global bleaching event, 75% of reefs around the globe were impacted (Hughes *et al.,* 2018).

Physiological and genotype variations among different algal symbionts have critical role in understanding the ecological resilience of coral reefs to thermal stress (Wham *et al.,* 2017; Manzello *et al.,* 2019). The Symbiodiniaceae species are ecologically diverse, exhibiting various associations with different coral hosts over large geographical scales, depths, seasons, and exposure (LaJeunesse *et al.,* 2010; Ziegler *et al.,* 2017). Their specific identities are strongly correlated with their host's susceptibility to ocean temperature. Therefore, affecting broad disparities in thermal tolerance among individual colonies and host species (Berkelmans and van Oppen 2006; Sampayo *et al.,* 2008; La Jeunesse *et al.,* 2010).

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Family Symbiodiniaceae consists of several genetically divergent lineages called clades. There are clades A through J currently reported (Pochon and Gates 2010; Nitschke *et al.,* 2020; Yorifuji *et al.,* 2021), with the clades A, B, C, and D as most commonly associated with corals (Pochon *et al.,* 2014). Some clades and subclades later were designated as genera which consist of numerous types. While some of the types were then characterized as species (LaJeunesse and Thornhill, 2011; LaJeunesse *et al.,* 2018). Recently, *Cladocopium* was reported as the most prevalent and widely distributed genus among the coral hosts (LaJeunesse *et al.,* 2010; Ziegler *et al.,* 2017). One member namely *Cladocopium thermopilum* showed strong tolerance to high temperatures (Hume *et al.,* 2015).

Coral reefs are important resources in Indonesia, supporting the reef fisheries and contributing to national food security. About 60% of animal protein consumption is derived from the fisheries (Dey *et al.,* 2005). However, most of the reefs are rapidly declining and threatened by anthropogenic activities (Burke *et al.,* 2012). Moreover, despite harboring 14% of the world total and being home of 590 described coral species (Wallace 2000), there were few reports on the *Symbiodinium* diversity in the Scleractinian corals from Indonesia (Hennige *et al.,* 2010; de Boer *et al.,* 2012; Roriris *et al.,* 2017; Wijayanti *et al.,* 2022). Several bleaching events, including the most severe one at 1997-1998 with coral mortality of nearly 90% (Azhar *et al.,* 1999) have been reported from Indonesian reefs. Another mass bleaching event was reported in 2016, causing mortality ranging from 25 to 50% of hard corals in various locations except in Raja Ampat (Habibi *et al.,* 2017). The impact of bleaching events can be more severe in Indonesia since most reefs are fringing reefs attached to the land with increasing development activities and receiving local stressors directly (Baum *et al.,* 2015). Moreover, corals that have experienced severe bleaching have lower Symbiodiniaceae density (Dosoky *et al.,* 2020).

This study investigates the genotype of Symbiodiniaceae of *Acroporidae, Poritidae, Pocilloporidae, Montastraeidae, Merulinidae, Agariciidae, Mussidae, Lobophyliidae, Oculinidae, Fungiidae, and Euphylliidae* from three different localities of Indonesia reefs, namely Panjang Island and Karimunjawa Archipelago at the Java Sea, and Tulamben Waters, Karangasem District, Bali. All locations represented heavily impacted areas by human anthropogenic activities mainly from tourism and fishery activities (Fafurida *et al.,* 2020; Munasik *et al.,* 2020) and experienced bleaching events (Wouthuyzen *et al.,* 2017).

Materials and Methods

Samples collection and sampling area

Samples were collected at Panjang Island and Karimunjawa Archipelago using SCUBA diving at depths of 1 to 3 m in Panjang Island, while samples from Karimunjawa Archipelago were collected at depths of 2 to 4 m of Cemara Kecil Island, Barakuda Beach of Kemujan Island, Sambangan Island, Bengkoang Island, Geleang Island, and Tanjung Gelam Beach of Karimunjawa Island (Figure 1). Samples from Tulamben Waters, Karangasem District, Bali were collected using scuba diving at depths of 3 and 10 m. The sample collection was done by applying the time-swim method (Raymundo *et al.,* 2008). All samples were collected under permission of the Karimunjawa National Park Authority (No. 1392/T.34/TU/ SIMAKSI/03/2020, No. 1505/T.34/TU/SIMAKSI/ 08/2021) and the Investment Agency and One Stop Service for the Bali Province and the Provincial Political and National Unity Agency (No. 070/1602/IZIN-C/DISPMPT). Small fragments of massive corals about 1-3 cm were collected using a hammer and chisel. Fragments of branching corals were cut using cutting pliers. All samples were then kept in individually labeled ziplock plastic containing 95% ethanol before further analysis. The total number of samples at each sampling location and the names of coral families are indicated in Table 1.

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DNA extraction, amplification and sequencing

Coral tissue including symbiotic algae was extracted following the procedures of LaJeunesse *et al.* (2004). DNA extraction from the tissue was carried out using a Geneaid Genomic DNA Mini Kit (Plant) following the manufacturer's procedures. The spin-column DNA extraction was utilized to obtain the algal DNA for amplification. Polymerase Chain Reaction (PCR) was performed on all extracted samples by amplifying the ITS-rDNA region gene. The PCR reaction consisted of: 1.25 µl of DNA template, 9.25 µl of ddH2O, 12.5 µl of Taq DNA Polymerase (Red Mix, Bioline, Meridian Bioscience, London, UK), and 1 µl of both primers (10 nm) with a total volume of 25 µl. The primers used were R18Sf-forward (5'-GAA AGT TTC ATG AAC CTT AT-3') and Sym28Sr-reverse (CTT GTR TGA CTT CAT GCT A-3'). Amplification was conducted with pre-denaturation conditions of 94°C (5 min), 35 cycles of denaturation of 94°C (45 sec), 51°C of annealing (45 sec), and 72°C of extension (1 min), followed by post extension of 72°C (7 min) (Yamashita and Koike 2013). All PCR products were then analyzed by electrophoresis in 1% agarose gel (FMC Bioproduct, Rockland, ME, USA) in 1x SB buffer and added 4 µl of Fluorescent DNA Stain (1^{st} Base) and run for 30 min (400 V; 100 mA).

Table 1. Number of corals collected and the sampling locations

No	Location	Family Name	Coral species	Number Colonies Collected	BLAST
1	Panjang Island, Jepara (-6° 34' 34.27", 110° 37' 53.04")	Acroporidae	*Acropora* sp.	9	4
		Acroporidae	*Montipora* sp.	4	3
		Poritidae	*Porites* sp.	5	3
		Acroporidae	*Stylophora* sp.	2	1
		Pocilloporidae	*Pocillopora* sp.	3	1
		Merulinidae	*Goniastrea* sp.	5	3
		Oculinidae	*Galaxea* sp.	2	0
		Agariciidae	*Pavona* sp.	1	1
		Mussiidae	*Favia* sp.	4	0
		Merulinidae	*Platygyra* sp.	2	0
		Merulinidae	*Favites* sp.	5	1
		Merulinidae	*Merulina* sp.	1	0
	Subtotal			43	16
2	Karimunjawa Archipelago, Jepara (5°40'39”- 5°55'00” and 110°05' 57”-110°31' 15”	Acroporidae	*Acropora muricata*	19	19
		Acroporidae	*Acropora* sp.	4	4
		Pocilloporidae	*Pocillopora* sp.	4	1
		Oculinidae	*Galaxea* sp.	4	4
		Mussiidae	*Favia* sp.	4	4
	Subtotal			35	30
3	National Marine Park Tulamben, Bali (-8° 16' 42.91", 115° 35' 48.58") Depth 3 m	Merulinidae	*Favites* sp.	2	1
		Acroporidae	*Acropora* sp.	3	1
		Acroporidae	*Isopora palifera*	1	0
		Acroporidae	*Montipora* sp.	3	2
		Montastraeidae	*Montastrea* sp.	3	0
		Poritidae	*Porites* sp.	4	2
		Mussiidae	*Favia* sp.	2	0
		Agariciidae	*Pavona* sp.	1	1
		Oculinidae	*Galaxea* sp.	1	0
		Pocilloporidae	*Pocillopora* sp.	1	0
		Merulinidae	*Platygyra* sp.	1	0
		Merulinidae	*Leptoria* sp.	1	1
		Merulinidae	*Goniastrea* sp.	1	1
	Subtotal			24	9
4	National Marine Park Tulamben, Bali (-8° 16' 42.91", 115° 35' 48.58") Depth 10 m	Acroporidae	*Montipora* sp.	5	1
		Acroporidae	*Stylophora* sp.	1	1
		Agariciidae	*Pachyseris* sp.	1	0
		Lobophylliidae	*Lobophyllia* sp.	1	1
		Merulinidae	*Leptoria* sp.	1	0
		Oculinidae	*Galaxea* sp.	1	1
		Poritidae	*Goniopora* sp.	1	0
		Merulinidae	*Platygyra* sp.	1	1
		Fungiidae	*Fungia* sp.	1	1
		Montastraeidae	*Montastrea* sp.	2	1
		Merulinidae	*Goniastrea* sp.	1	0
		Euphylliidae	*Euphyllia* sp.	1	1
	Sub Total			17	8
	TOTAL			119	63

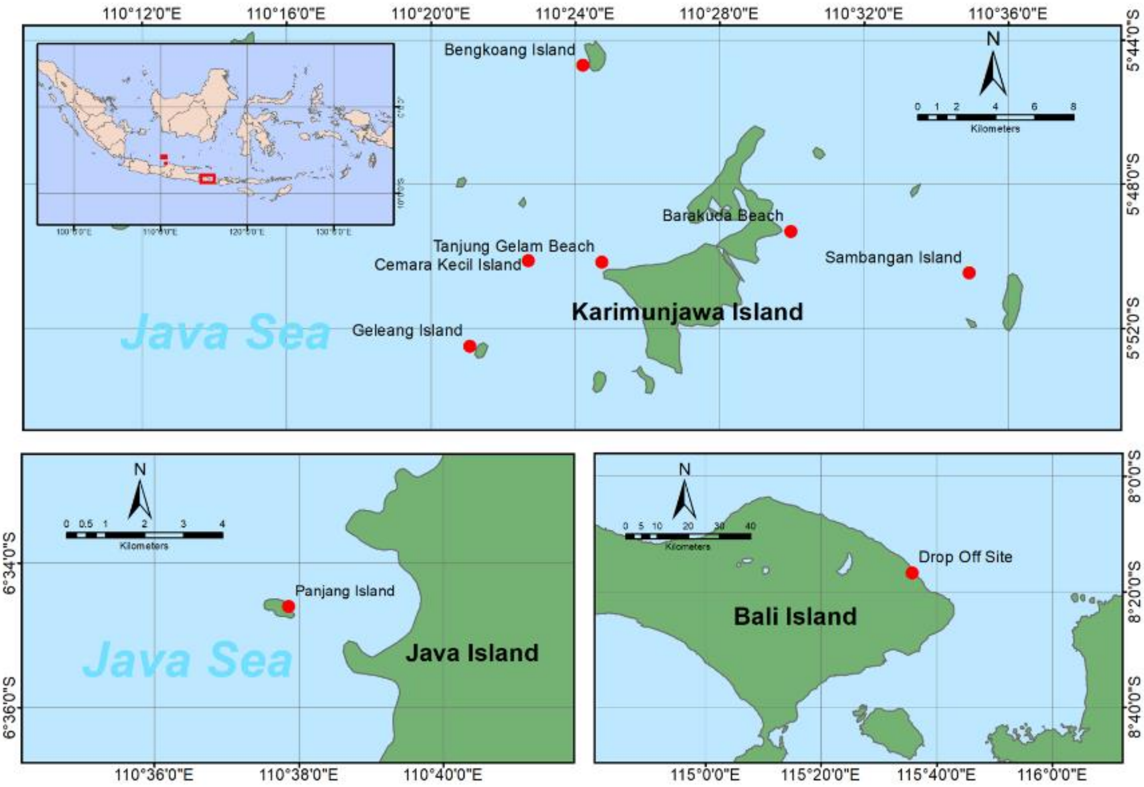

Figure 1. Sampling locations at Panjang Island, Karimunjawa Archipelago, and Bali Island

The gel was then visualized under a UV Transilluminator (Uvitec Fire Reader, Cambridge, UK). All amplified samples were then sent to PT. Genetika Science (Jakarta) for sequencing. Sequences were conducted using ABI 3730xl 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 Institute for Health USA (www.ncbi.nlm.nih.gov) searching was applied to confirm the sequence obtained against the gene database. Alignment of the results was carried out using CLUSTAL W (Thompson *et al.,* 1994). The phylogenetic tree was constructed using the Maximum likelihood method with a 2-parameter Kimura model (Loh *et al.,* 2001). Bootstrap analysis with 1000 replicates for the Maximum likelihood was performed.

Result and Discussion

In total, there were 63 sequences out of 119 coral colonies from 16 genera obtained from Panjang Island, Karimunjawa Archipelago, and Tulamben waters, Bali. The length of the amplified ITS-rDNA region was approximately 600 bp, regardless of the type of coral host. The homology analysis using BLAST searching of ITS-rDNA region sequences showed that samples matched to *Cladocopium* sp. and *Durusdinium* sp. with similarities ranging between 96.79-100% (Table 2–4).

All nucleotide sequences have been deposited in the Genbank database under accession numbers MT831949 – MT831966 for the samples collected from Cemara Kecil and Barakuda Beach of Karimunjawa Archipelago, and ON705574 – ON705636 for the rest of the samples. *Cladocopium* dominated symbiont type of coral hosts collected from various islands of Karimunjawa Archipelago and Tulamben Waters, Bali. While only 50% (n=16) of corals collected from Panjang Island have *Cladocopium* with the rest of the samples hosting *Durusdinium* (Table 2 – 4).

Phylogenetic tree and genetic distance

The Symbiodiniaceae sequences obtained from the coral hosts were analyzed to understand the relationship among sampling locations by reconstructing a phylogenetic tree. *Ansanella granifera*, a member of Dinophyceae, represents the outgroup, while several members of *Cladocopium* were used as the in-group (Figure 2).

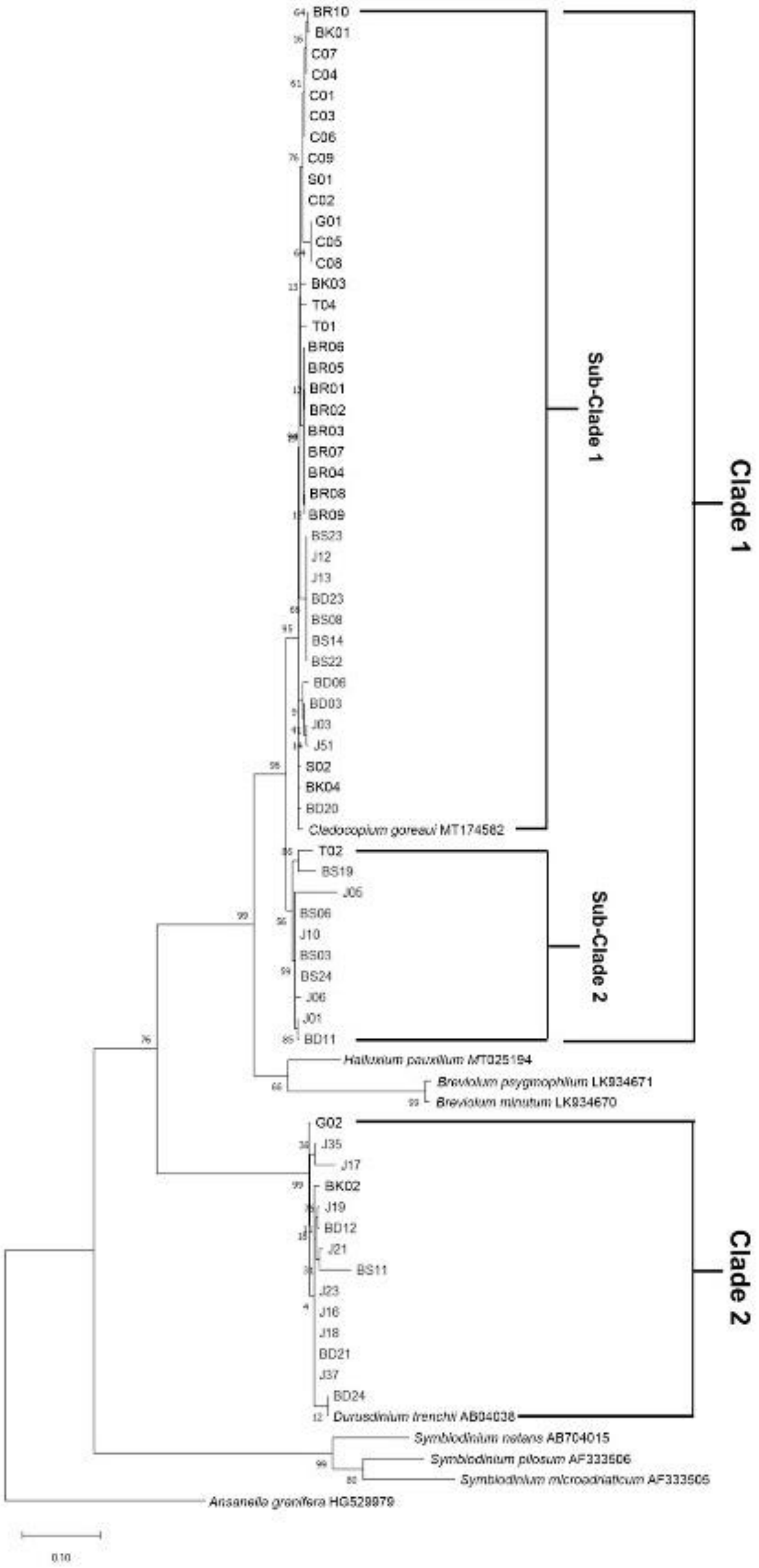

Figure 2. Maximum likelihood tree based on the ITS-rDNA sequence data showing the relationships of Symbiodiniaceae clade with the most closely related Symbiodiniaceae recorded in the GenBank database. The Symbiodiniaceae were grouped into 2 clades. *Ansanella granifera*, a member of Dinophyceae, represents the outgroup.

The Maximum likelihood analysis of Symbiodiniaceae showed that the algal symbiont types from the Scleractinian samples formed two clades, *i.e.* Clade 1 and Clade 2, with Clade 1 consisting of two subclades. All coral hosts that are grouped in the subclade1 have *Cladocopium* sp. 2486705 as their symbiont. The host corals are *Acropora* sp., *Pocillopora* sp., *Favia* sp., *Goniastrea* sp., *Platygyra* sp., *Favites* sp., *Leptoria* sp., *Stylophora* sp., *Lobophyllia* sp., *Galaxea* sp., and *Montastrea* sp. which were collected from Tulamben waters (8 samples), Karimunjawa Archipelago (8 samples) and Panjang island (4 samples). While *Galaxea* sp., *Porites* sp., *Montipora* sp., and *Echinopora* sp. collected from Tulamben (5 samples), Panjang Island (4 samples) and Tanjung Gelam Beach (1 sample) were associated with *Cladocopium* sp. and grouped in the subclade 2. *Durusdinium* sp. was found associated with *Galaxea* sp., *Goniastrea* sp., *Acropora* sp., *Montipora* sp., *Lobophyllia* sp., *Pavona* sp., *Pocillopora* sp., *Fungia* sp., and are grouped in the Clade 2 (Figure 2.).

Galaxea sp. was the only coral host that harbored *Cladocopium* sp. 2486705, *Cladocopium* sp. and *Durusdinium* sp. However, there is no specific location for different types of symbionts. *Galaxea* sp. collected from Sambangan hosted *Cladocopium* sp. 2486705, while *Galaxea* sp. collected from Tanjung Gelam Beach had *Cladocopium* sp. as the algal symbiont. *Galaxea* sp. collected from Bengkoang and Geleang Islands, Karimunjawa Archipelago and Tulamben waters, Bali hosted *Durusdinium* sp. (Figure 2.).

Among the 16 genera of 63 coral colonies from three localities at Java Sea and Bali, only two symbiont types were identified, i.e. *Cladocopium* and *Durusdinium* (Table 2–4). The results were consistent with previous studies conducted at Wakatobi Islands, South East Sulawesi (Hennige *et al.,* 2010) and Biawak Island, West Java Province (Roriris *et al.,* 2017) which observed the Symbiodiniaceae clade C in the coral host samples. *Cladocopium* was previously known as *Symbiodinium* clade C, while *Durusdinium* was recognized as clade D, both being the most common genera hosted by corals, especially in Pacific region (LaJeunesse, 2005). *Cladocopium* and *Durusdinium* were also reported as the most abundance symbiont types from coral hosts in Singapore (Smith *et al.,* 2020), Gulf of Thailand (Chankong *et al.,* 2020) and West Island, Sanya, China (Wang *et al.,* 2021).

Reports suggested that corals hosting thermally tolerant Symbiodiniaceae show ability to adapt to high sea surface temperatures. Symbiodiniaceae have been characterized for their ability to tolerate and sensitivity to thermal stress (Sampayo *et al.,* 2008; LaJeunesse *et al.,* 2010; Silverstein *et al.,* 2015; Swain *et al.,* 2017). Several symbiotic subclades have been reported to be heat-sensitive (for example C3, C7, and A13), while some members of *Cladocopium* and *Durusdinium* are considered to be heat-tolerant (Bhagooli, 2009, 2010; Hume *et al.,* 2015; Silverstein *et al.,* 2015; Swain *et al.,* 2017).

The genus *Cladocopium* is known to have the most species-rich members (LaJeunesse *et al.*, 2018), likely comprising over 100 species (Thornhill *et al.*, 2014). *Cladocopium* are broadly distributed and present in the Pacific, Atlantic, and Indian Oceans (Nitschke *et al.*, 2022). The genus is ecologically abundant in various coral reef communities in the Indo-Pacific (Leveque *et al.*, 2019; Lim *et al.*, 2019) as well as the genus *Durusdinium* which is also commonly found in the Pacific, Atlantic, and Indian Oceans (Nitschke *et al.*, 2022). *Cladocopium* is found in various symbiont types including scleractinian corals; Hexacorallia (Rowan and Powers, 1991), Hydrozoa and Octocorallia (LaJeunesse *et al.*, 2010), and Scyphozoa (Klein *et al.*, 2017), at different depth, and habitats. While *Durusdinium* has been reported to form symbioses with scleractinian corals, Hexacorallia (Wham *et al.*, 2011), Octocorallia (Goulet *et al.*, 2008a) and scyphozoans (Lampert *et al.*, 2012).

There are 5 species currently described namely *Cladocopium goreaui*, *C. thermopilum, C. latusorum, C. pocilloporum*, and *C. infistulum* (which is reported as the symbiont of bivalve mollusc) (Lee *et al.*, 2020). Four species namely *Durusdinium trenchii; D. glynnii; D. boreum; and D. eurythalpos* are currently described. *D. trenchii* is reported as the most extensively studied in the Indo-Pacific for its thermal tolerance and persistence after coral bleaching events (Berkelmans and Van Oppen, 2006). The members of the genera are also reported to have a high thermal tolerance (Howells *et al.*, 2020; Lee *et al.*, 2020), especially for *Durusdinium* can be categorized as 'extremophiles' for their ability to adapt to extreme environments (Nitschke *et al.*, 2022).

The number of coral hosts that harbored the *Durusdinium* was lower compared to the corals that hosted the *Cladocopium* (Table 2–4). Similarly, *Durusdinium* was reported to be less abundant on the reefs across Singapore, despite its crucial role in the existence of the reef (Smith *et al.,* 2020). Our results showed that 50% (n=16) of samples collected from Panjang Island harbored *Durusdinium* (Table 2.). The reefs of Panjang Island have long experienced extensive disturbance (Sabdono *et al.,* 2014; Suryono *et al.,* 2021). LaJeunesse *et al.* (2018) suggested that the *Durusdinium* is usually found in shallow corals exposed to extreme environmental pressures.

Table 2. Homology analyses of Symbiodiniaceae diversity from Panjang Island, Jepara

Samples code	Host	BLAST Identification	Base pair	Query Cover (%)	Identify (%)	Accession Code
J01	*Echinopora* sp.	*Cladocopium* sp.	543	100	99.69	MK775980
J03	*Acropora* sp.	*Cladocopium* sp. 2486705	656	100	99.85	MK654289
J05	*Porites* sp.	*Cladocopium* sp.	637	99	99.05	MK775980
J06	*Stylopora* sp.	*Cladocopium* sp.	658	99	99.39	MK775980
J10	*Porites* sp.	*Cladocopium* sp.	658	100	99.85	MK775980
J12	*Acropora* sp.	*Cladocopium* sp. 2486705	661	100	98.94	MN654289
J13	*Acropora* sp.	*Cladocopium* sp. 2486705	656	100	99.54	MN654289
J16	*Pocillopora* sp.	*Durusdinium* sp.	661	100	100	MN891786
J17	*Acropora* sp.	*Durusdinium* sp.	659	99	96.79	MN891786
J18	*Pavona* sp.	*Durusdinium* sp.	660	100	100	MN891786
J19	*Montipora* sp.	*Durusdinium* sp.	661	100	99.39	MN891786
J21	*Montipora* sp.	*Durusdinium* sp.	664	100	99.40	MN891786
J23	*Goniastrea* sp.	*Durusdinium* sp.	664	100	100	MN891786
J35	*Goniastrea* sp	*Durusdinium* sp.	672	99	98.96	MN891779
J37	*Goniastrea* sp	*Durusdinium* sp.	664	100	100	MN891786
J51	*Favites* sp.	*Cladocopium* sp. 2486705	658	100	99.54	MN654289

Tabel 3. Homology analyses of Symbiodiniaceae diversity from Karimunjawa Archipelago, S: Sambangan Island; BK: Bengkoang Island; G: Geleang Island; T: Tanjung Gelam Beach; C, Cemara Kecil; BR, Barakuda

Samples Code	Host	BLAST Identification	Base pair	Query Cover (%)	Identify (%)	Accession Code
S02	*Galaxea* sp.	*Cladocopium* sp. 2486705	649	99	100	MN654289
S01	*Acropora* sp.	*Cladocopium* sp. 2486705	681	99	98.67	MN654291
BK04	*Favia sp.*	*Cladocopium* sp. 2486705	655	100	99.69	MN654289
BK01	*Acropora* sp.	*Cladocopium* sp. 2486705	668	100	98.95	MN654291
G01	*Acropora* sp.	*Cladocopium* sp. 2486705	656	100	99.54	MN654289
T04	*Favia* sp.	*Cladocopium* sp. 2486705	669	100	98.65	MN654291
T01	*Acropora* sp.	*Cladocopium* sp. 2486705	677	99	98.22	MN654291
BK03	*Pocillopora* sp.	*Cladocopium* sp. 2486705	665	98	97.56	MN654289
T02	*Galaxea* sp.	*Cladocopium* sp.	647	99	97.37	MK775980
G02	*Galaxea* sp.	*Durusdinium* sp.	628	99	99.20	MN891782
BK02	*Galaxea* sp.	*Durusdinium* sp.	651	99	99.54	MN891786
C01	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
C02	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.97	MN654291
C03	*Acropora* sp.	*Cladocopium* sp. 2486705	680	99	98.67	MN654291
C04	*Acropora* sp.	*Cladocopium* sp. 2486705	679	99	98.82	MN654240
C05	*Acropora* sp.	*Cladocopium* sp. 2486705	677	99	99.11	MN654291
BR01	*Acropora* sp.	*Cladocopium* sp. 2486705	677	99	98.96	MN654291
BR02	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR03	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR04	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR05	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR06	*Acropora* sp.	*Cladocopium* sp. 2486705	677	99	98.96	MN654291
BR07	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR08	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR09	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.82	MN654291
BR10	*Acropora* sp.	*Cladocopium* sp. 2486705	680	98	98.66	MN654291
C06	*Acropora* sp.	*Cladocopium* sp. 2486705	689	98	98.96	MN654291
C07	*Acropora* sp.	*Cladocopium* sp. 2486705	682	98	98.81	MN654291
C08	*Acropora* sp.	*Cladocopium* sp. 2486705	683	98	98.24	MN654291
C09	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	98.97	MN654291

Table 4. Homology analyses of Symbiodiniaceae diversity of Tulamben, Bali, BS : Bali 3m depth; BD : Bali 10 m depth

Samples code	Host	BLAST Identification	Base pair	Query Cover (%)	Identify (%)	Accession Code
BS03	*Montipora* sp.	*Cladocopium* sp.	670	99	99.85	MK775980
BS06	*Porites* sp.	*Cladocopium* sp.	679	98	100	MK775980
BS08	*Favites* sp.	*Cladocopium* sp. 2486705	679	99	99.11	MN654291
BS11	*Pavona* sp.	*Durusdinium* sp.	649	90	99.14	MN891786
BS14	*Acropora* sp.	*Cladocopium* sp. 2486705	678	99	99.26	MN654291
BS19	*Porites* sp.	*Cladocopium* sp.	671	99	97.02	MK775980
BS22	*Leptoria* sp.	*Cladocopium* sp. 2486705	669	99	99.25	MN654291
BS23	*Goniastrea* sp.	*Cladocopium* sp. 2486705	679	99	99.11	MN654291
BS24	*Montipora* sp.	*Cladocopium* sp.	671	99	99.55	MK775980
BD03	*Lobophyllia* sp.	*Cladocopium* sp. 2486705	678	99	99.26	MN654291
BD06	*Stylopora* sp.	*Cladocopium* sp. 2486705	678	99	99.26	MN654291
BD11	*Montipora* sp.	*Cladocopium* sp.	566	98	99.69	MK775980
BD12	*Lobophyllia* sp.	*Durusdinium* sp.	682	99	99.27	MN891779
BD20	*Montrastrea sp.*	*Cladocopium* sp. 2486705	674	99	98.38	MN654291
BD21	*Galaxea* sp.	*Durusdinium* sp.	683	99	99.85	MN891779
BD23	*Platygyra* sp.	*Cladocopium* sp. 2486705	669	99	99.25	MN654291
BD24	*Fungia* sp.	*Durusdinium* sp.	685	99	99.56	MN891779

Members of *Durusdinium* consist of stress-tolerant species (LaJeunesse *et al.,* 2018), which show the ability to adapt to a highly impacted and turbid environment (Smith *et al.,* 2020).

The results showed that most *Acropora* sp. hosted *Cladocopium* as their algal symbiont, except for one sample from Panjang Island. While *Pocillopora* sp, *Montipora* sp, *Goniastrea* sp., and *Lobophyllia* sp. have either *Cladocopium* or *Durusdinium* as symbionts. All *Porites* sp. collected from the sampling location harbored *Cladocopium* while all *Pavona* sp. hosted *Durusdinium*. Only *Galaxea* sp. that hosted *Durusdinium*, *Cladocopium*, and *Cladocopium* sp. 2486705 (Table 2–4). Tong *et al. (*2017) found that *Galaxea fascicularis* colonies with Durusdinium as their dominant algal symbiont showed higher thermal stress resistance than *Montipora* which hosted *Cladocopium*. Qin *et al. (*2019) suggested that corals with closer phylogenetic relationships seem to associate with similar dominant Symbiodiniaceae types which imply the host played a crucial role in controlling their symbiont types (Sampayo *et al.,* 2008; Putnam *et al.,* 2012). Therefore, more adapted corals are most likely associated with heat-tolerant Symbiodiniaceae. For instance, in the South China Sea, the coral species that become dominant are likely associated with heat-tolerant *Durusdinium* such as *Pocillopora verrucosa* (Qin *et al.,* 2019). However, *Acropora* spp. which mostly harboured *Cladocopium* as their algal symbiont is the dominant coral species of our sampling location (Tomascik *et al.,* 1997) except in Panjang Island which are dominated by massive corals such as *Porites* and *Favites* (Munasik *et al.,* 2012). Although *Acropora* and *Pocillopora* species have high symbiotic flexibility, the corals are more stress-sensitive than massive *Porites* corals that have high fidelity to a single symbiont type (Putnam *et al.,* 2012). More studies are needed to address the question of the mechanism relating to how corals adapt to environmental changes.

Conclusion

This study provides more data on the diversity of the algal symbiont type of coral host from Indonesia which currently is scarce. This study identified a predominance of *Cladocopium* among coral hosts collected from Panjang Island and six islands of Karimunjawa Archipelago of the Java Sea and Tulamben Waters, Bali. *Durusdinium* was present in a lower number of samples. The use of ITS ribosomal DNA markers helps to observe the genetic diversity of the Symbiodiniaceae. However, developing new molecular markers such as psbA coupled with more powerful genetic data such as those generated by next-generation sequencing needs to be considered to obtain more detailed results.

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