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Coral *Echinoporalamellosa* Hosts Multiple Clades of Symbionts in western Alas Strait, Indonesia

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Abstract. Coral symbiont diversity is one of the most important factors in coral resistance and coral reef resilience to bleaching. Many corals host only one symbiont clade, thus many of them vulnerable to climate change induced bleaching. Corals in Alas Strait have survived from several bleaching events. The present study aimed to look at genetic factors that potentially contributed to the survival of *Echinoporalamellosa* coral. Previously, *E. lamellosa* was reported to host only one clade of symbiont, either in Pacific or Indian Oceans. Here we provide new evidence on the ability of *E. lamellosa* in hosting multiple clades of symbionts. The symbiont rDNA was extracted from small *E. lamellosa* fragments and amplified by targeting 'the non-coding gene region' of ITS1-5.8S-ITS2. The clustering technique was used to determine the clade type of symbiont, by constructing the phylogenetic tree consist of the sequenced samples and downloaded sequences from GenBank NCBI as comparison. The constructed phylogenetic tree was based on *Maximum Likelihood* (ML) with *bootstrap* value 1000× to get the best result. The result shows the symbiont in *E. lamellosa* tissue belonged to clades B and C. The combination of the two symbiont clades has never been recorded in previous studies, and it is very likely to have contributed to coral survival from bleaching events.

INTRODUCTION

Genetic diversity of coral symbiont has important contribution to coral survival in coping with thermal-induced bleaching [1; 2]. Genetic diversity evolved through evolutionary processes and created symbiont clades with different adaptable physiological features [3-5]. Adaptive bleaching hypothesis suggests that corals are able to host new symbionts from more adaptable clades if the corals survived from the bleaching event [6]. Coral that hosts symbionts from thermal-tolerant clades tend to survive the next bleaching event [7; 8]. *El Niño Southern Oscillation* (ENSO) has caused the Pacific Ocean sea temperature to rise 2 to 3°C in this last decade [9; 10], forcing the corals to host thermal-tolerant clades, such as clades A or D [11-13], and lowering the clade variation in result.

Coral bleaching in 1998, 2010, and 2016 have caused mass mortality of corals worldwide [14; 15; 16]. The most severe coral bleaching happened in 1997-1998. At that time, the sea temperature has risen by approximately 2°C globally, causing the death of 16% of corals worldwide [17; 18]. In 2010 and 2016, bleaching reoccurred and caused corals mass mortality in the Caribbean [19], Indian Ocean [20; 21], South-East Asia (including Indonesia) [22; 23], and Australia [24; 25].

Corals of Lombok Island and nearby islets were also impacted by bleaching. During the bleaching event in 2016, at least 69% of corals in GiliMatra (GiliMeno, GiliTrawangan, and Gili Air), North Lombok turned to white [26] and the coral cover in Sekotong Bay, West Lombok was significantly reduced to 18% [27]. Bleaching also occurred in eastern Lombok waters, especially in the MPA (Marine Protected Area) of GiliSulat-Lawang. About 60.06% of corals in no fishing zones of the MPA was found bleached, another 63.89% of bleached corals was found in the fisheries zone, and only 12.99% coral bleached in outer region of the MPA (northern part of GiliLawang) [28].

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However, although the MPA of GiliSulat-Lawang was impacted by the bleaching, our observation shows that most coral in the nearest islet, GiliPetagan, survived from the 2016 bleaching event. There are 3 possible explanations/hypotheses of their survival factors, 1) the involvement of heat-tolerant symbiont which are commonly found after bleaching events [8; 13], 2) the presence of mangrove vegetation as shelter [29; 30], or 3) turbidity that provides shade protection for corals to survive against UV light [31; 32]. The present study aims to test the first hypothesis that corals of Alas Strat hosts heat resistant symbionts in their tissue.

MATERIALS AND METHODS

Sampling

All sampling was conducted in GiliPetagan (a small islet on western side of Alas Strait) in September 2019 (Figure 1). Fragments of *Echinoporalamellosa* (2-3 cm² in size) were collected from 30 large colonies at ± 5 m depth lined the reef slope. The fragments were then preserved in 95% ethanol and stored in a cold chamber. To reduce the chance of sampling clones, each sampling colonies of *E. lamellosa* were photographed and taken at least 3 m apart within the location.

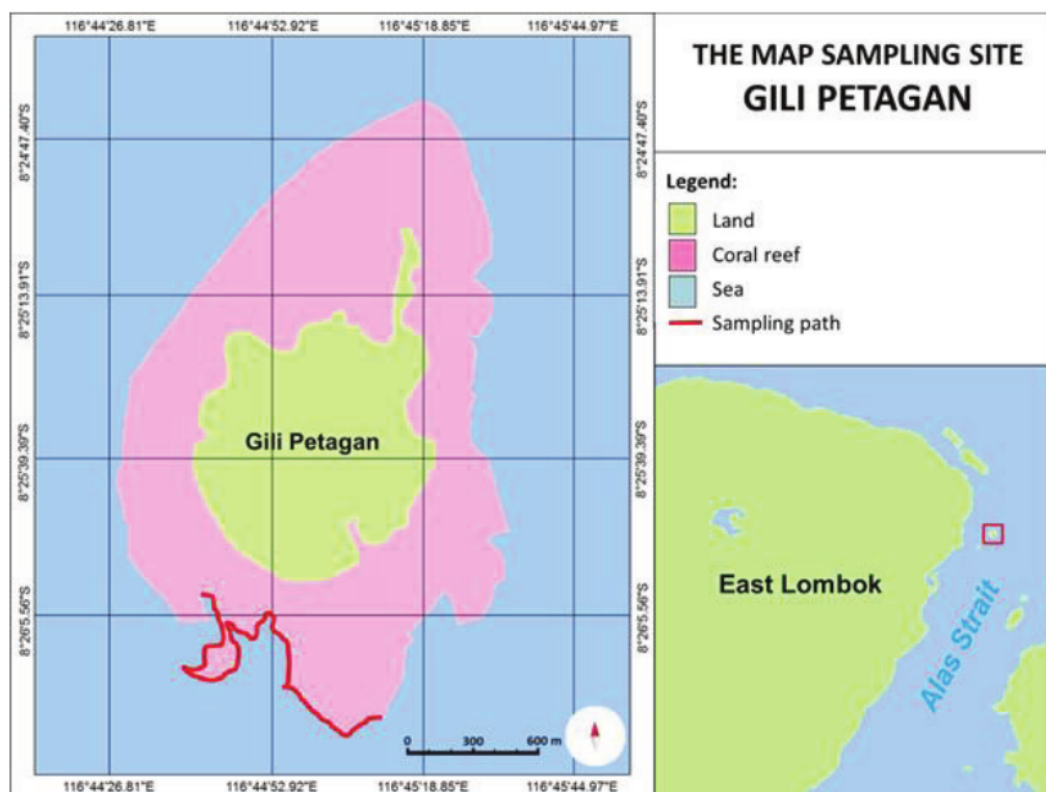


FIGURE 1. The map of sampling site in GiliPetagan, Alas Strait

DNA Extraction

The whole genomic DNA of both host and symbiont was extracted from pulverized and sterilized *Echinoporalamellosa* tissue by using the DNeasy™ Blood and Tissue Kit (Qiagen®), following the manufacturer's protocol (Qiagen® manufacturer protocol for extracting DNA of tissue and blood) with 12 hours pre-incubation. Multiple centrifugation steps were done to separate the host and symbiont DNA. A check of extracted symbiont DNA quality was performed on a 1% agarose gel. The samples with low quality of extracted DNA were discarded and re-extracted.

Amplification and Preparation for Sequencing

The ITS1-5.8S-ITS2 region (whole ITS region) of *Symbiodinium* was amplified by using forward primer ITS1 (5'-CTTGTTTCATTAGAGGAAGTAA-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), following [33] with minor modifications. Each primer was added to a reaction mixture, which contained 12.5 µL TaqDNA polymerase (MyTaq™ HS Red Mix, Bioline®), 9 µL ddH₂O (SuperPure nuclease-free water, BioScience®) and 2 µL DNA template. The amplification process was performed in a pEqSTAR™ Thermal Cycler (PEQLAB®) with the thermal profile: 35 cycles of 45s at 94°C, 30s at 47°C and 1 minute at 72°C.

The quality of amplicon (the sample product of amplification process) was checked through agarose gel electrophoresis. The good quality samples were identified by clear and thick bands. Only good quality samples with different size of bands were sent to 1st Base-Asia, Malaysia (<http://www.base-asia.com>) for further sequencing.

Sequence Analysis

Sequence analysis was done based on [34]. The DNA barcoding method was carried out by using BLAST feature in NCBI to confirm the sequence as true symbionts. Editing process and alignment of the sample sequence with comparison sequence from GenBank were done by MEGA V6.0. Clades A, B, C and D of *Symbiodinium* are associated with the coral colonies worldwide and the rest of the clades are commonly found as symbionts of other invertebrates [4; 35]. So, only the sequences of *Symbiodinium* clades A, B, C and D were retrieved from GenBank as a comparison sequence in constructing the phylogenetic tree. Bootstrapped Maximum Likelihood tree based on Kimura 2-parameter distance matrix, with 1000 replication. The constructed phylogenetic tree and the determination of outgroup were matched with the visual photograph of electrophoresis bands to validate the result.

RESULTS

Visual Data of Electrophoresis Bands and The Sequence Confirmation

The electrophoresis result showed two different sizes of bands, approximately 600 to 750bp (Figure 2). Based on sequence confirmation by using BLAST feature in NCBI, all samples were confirmed as *Symbiodinium* (Table 1). The result indicated that there is a possible variation among the two groups of different sized bands.

TABLE 1.The detail information of the confirmed symbiont sequence

Sample Code	Nucleotide Size	Confirmed ID [*]	Query Cover [*]	%ID [*]
A	639bp	<i>Symbiodinium</i> sp. XiaodonghaiGF	97%	99.52%
B	702bp	<i>Symbiodinium</i> sp. 5B-J	84%	92.05%

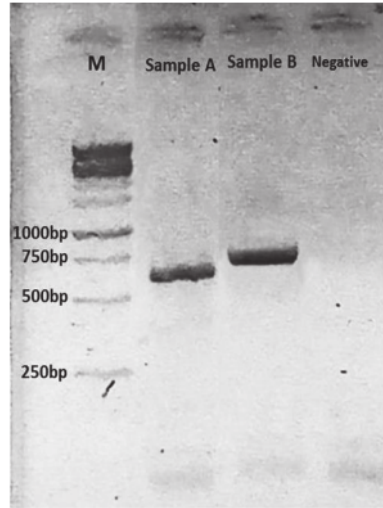


FIGURE 2.Two different size of band, indicating the variation among the samples

The Constructed Phylogenetic Tree

The clustering result performed through phylogenetic tree construction shows the sample belonging to clade B and C, as shown in Figure 3. The tree shows the small value of bar scale, indicating that the divergence value among the evolved sequences were low and became a strong justification to determine the outgroup. The high value of bootstrap (>90%) in every nodes validates the reliability of the constructed tree.

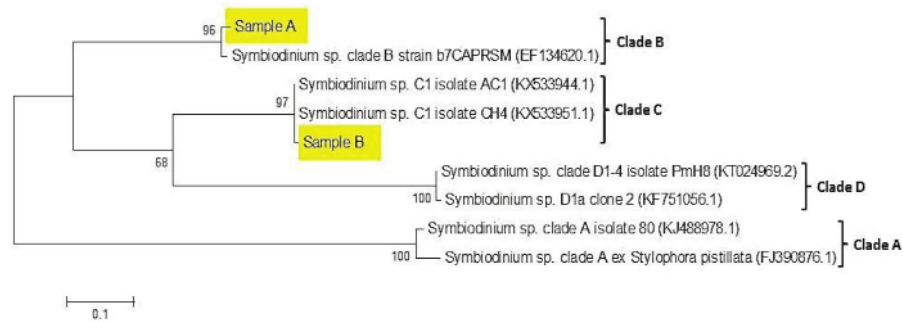


FIGURE 3.The constructed phylogenetic tree shows the samples belong to clade B and C

DISCUSSION

This study found that *Echinopora lamellosa* hosts clades B and C symbionts. Surprisingly, previous studies [36; 37; 38] reported that *E. lamellosa* only hosts clade C symbiont (as shown in Table 2.). Although this study provides preliminary information about a phenomenon that has never been recorded before, this study cannot provide an overview of the density ratio of zooxanthellae clades B and C in *E. lamellosa* tissue and also unable to generalize that *E. lamellosa* host only clades B and C in Alas Strait. In the other hand, the results of this study mean to prove the ability of *E. lamellosa* to host more than one type of symbiont clade and also confirm the clade symbionts that are living in *E. lamellosa* tissue.

TABLE 2. The comparison of *Symbiodinium* clade found in *E. lamellosa* based on previous and present studies

Author	Sampling site	Amplified region (marker)	Symbiodinium clade
Lohet <i>et al</i> (2002)	Indian Ocean and Northern Pacific	18S and 28S	C
LaJeunesse <i>et al</i> (2004)	Northern and Southern Pacific	ITS 2	C40, C3, C40b
Silverstein <i>et al</i> (2011)	Ningaloo Reef, Western Australia	ITS 2	C21, C40
Present study	Alas Strait, Indonesia	ITS 1-5.8S-ITS 2 region	B and C

The ability of *E. lamellosa* corals to host zooxanthellae clades B and C is an advantage of adaptation to survive in nature. The phenomenon of several *Symbiodinium* clades inhabiting single coral species generally occurs in the photic zone [39; 40; 41]. However, only about 23% of all coral species have the ability to host more than one *Symbiodinium* clade, while 77% others have high specificity for hosting symbionts [42]. Corals achieve the physiological advantages against the environmental stress when partnering with functionally diverse symbionts, and this ability is only available in corals that can perform associations with multiple *Symbiodinium* clades [43; 44]. The mutualistic relationship of coral-symbionts enables corals to utilize their symbiont physiological abilities to support their viability as host, as reported to occur in coral *Stylophora pistillata* [45]. All of these mechanisms may form the coral adaptation and survival in facing the menace of the environmental stress in nature. In addition, corals can survive the environmental stress by changing their symbiont with more adaptive type of symbionts [6], and this ability is not possessed by corals that can only host a single clade of symbiont [43].

Clade B symbionts are commonly known as coral symbionts of the tropic and subtropical regions of the Atlantic Ocean and Caribbean waters [46-49]. Clade B has never been found before as either coral's or other invertebrate's symbiont in the Indian Ocean [50; 51; 52] and rarely found as coral endosymbiont in the Pacific [53; 54]. The results of this study become one of the earliest records of the presence of clade B symbiont in the territory of Indonesia, apart from a study which reported the presence of clade B symbiont in *Acropora* sp. and *Goniastrea* sp. at Jepara waters [55]. Based on wider geographical coverage, this study also supports the data on the distribution of clade B as a coral symbiont in the Asia Pacific region in addition to previous studies conducted in Korean waters [53] and in French Polynesia [54].

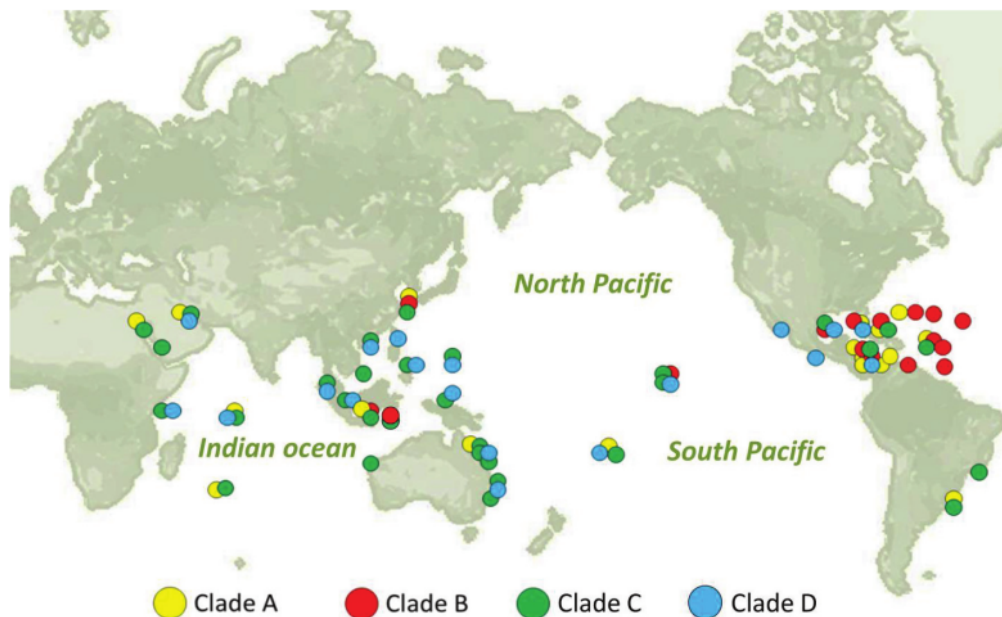


FIGURE 4. An updated of geographical distribution of coral symbiont clade (including the result of present study)

The symbiosis between clade B symbiont and hermatypic corals is often found in temperate areas and appears to be associated with lower water temperatures [53]. LaJeunesse et al. [56] revealed that the emergence of clade B symbiont is closely related to the phenomenon of *cold bleaching*, a type of coral bleaching caused by extremely cold temperature. However, this fact cannot be used to explain the presence of clade B in GiliPetagan. Based on the record, the coldest temperature of waters around the island of Lombok that was recorded at 25° C, occurred throughout July 2012 [57], whereas *cold bleaching* occurs when the water temperature dropped to 12° C [58], so a chance for *cold bleaching* to occur in this region is very small. In contrast, [59] found that the presence of clade B symbiont correlates with water quality and turbidity. This is the most possible explanation regarding to the presence of clade B in GiliPetagan, because the sampling area was always turbid caused by the mangrove vegetation nearby. This also can lead to the turbidity hypothesis [31; 32] (as we mentioned in introduction), but still need to be deeply elaborated.

Symbiodinium clade C are indeed a generalist symbiont of hermatypic corals and has the widest distribution area compared to the other clades [60; 61]. *Symbiodinium* clade C contribute to the acceleration of coral polyp growth 2-3 times faster compared to other clade zooxanthellae [62; 11], however, most of clade C phylotype are not considered to have good physiological abilities to support the host's viability against UV radiation and high seawater temperatures [63]. Clade C symbiont is the most vulnerable symbiont against the extreme changing of water temperature, especially subclade C1 [64]. Only several phylotypes, such as subclade C3 and C15 are known to have a good adaptation in dealing with the extreme rising of water temperatures and eutrophication [64; 65]. Corals that survived bleaching generally replace their symbionts with clades A and D [11-13], which are more resistant to the rising of water temperatures up to 33°C or against pollution [66-68].

The results of this study are contradictory to previous studies [8; 11; 69], which reported that surviving corals from bleaching will form a new symbiotic relation with more adaptive clades. No clade D but local strain symbionts were found in *E. lamellosa* after bleaching. This result was similar to Hume et al. [70], who found thermos-tolerant local-strain symbiont dominating post-bleaching corals in Arabian Gulf, without any presence of clade D. The

presence of the clade B symbiont in *E. lamellosa* also suggests the contribution of rare symbiont to coral resilience [71]. Another explanation that might be the cause of the absence of the clade D in *E. lamellosa* after bleaching is because the *E. lamellosa* and their symbiont (clade B and C) in Gili Petagan are living in the edge of their acclamatory potential so that the corals did not undergo adaptive bleaching [65].

The results suggest that the horizontal transmission may contribute to the formation of *E. lamellosa* colonies in Gili Petagan. The pattern of mass water flow distribution through Alas Strait can be a vector of coral polyps that was infected by zooxanthellae (algal symbiont) from other places. It has been reported that water flow has a major influence in carrying zooxanthellae-infected coral planula (horizontal transmission) in an open system [72; 73]. Corals can also obtain various symbionts from their environment through the horizontal transmission mechanism and the process occurs quickly [74]. In addition, corals may inherit the symbiont from their parent. Some corals can get the symbiont from both mechanisms. Coral *Stylophora pistillata* in the Red Sea was reported to have its symbiont through horizontal and vertical transmission mechanisms [45]. This explanation also correlates with the Adaptive Bleaching hypothesis stated by Buddemeier et al. [6]. Fluctuations of the environmental variability largely determine the consistency of symbiont development in coral tissue [75; 46]. However, the assumptions that have been stated cannot be used to make a conclusion about the origin of clade B and C symbionts of *E. lamellosa* in Gili Petagan. Further genetic analysis of other coral species at different depths and data of currents speed and direction, both locally in of Gili Petagan and in a wider geographical area need to be studied, so that the presence of clades B and C in coral *E. lamellosa* can be deeply understood.

CONCLUSION

Coral colonies *Echinopora lamellosa* hosting multiple clades has never been reported in previous studies. Our study provides a preliminary study of clades B and C as symbionts in *E. lamellosa* of western Alas Strait, proving the ability of this coral species to host multiple clades. Additionally, the presence of clade B in this study is a new record in the Indian Ocean. Clades B and C contribution to *E. lamellosa* survival in bleaching disasters is still unclear, but it is very likely that the rare clade B contributed to coral survival from previous bleaching events.

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