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Abstract. Karimunjawa is one of the earliest marine parks in Indonesia. Karimunjawa National Park (KNP) was designated as a marine conservation area to conserve marine resources from destructive fishing activities. Scleractinian corals in the genus Acropora are among the most dominant distributed in the KNPs, including the species of *Acropora hyacinthus*. Here, we present a comprehensive analysis of intra- and interspecific COI variabilities in *A. hyacinthus* to analyze genetic diversity and to describe the kinship relationship of the coral between 5 localities of the reefs. Genetic marker Cytochrome Oxidase I of the mitochondrial genome DNA (mtDNA) was used to analyze genetic diversity. Reconstruction of phylogenetic tree and genetic diversity were made by using software MEGA 5.05 (Molecular Evolutionary Genetics Analysis). The results indicate corals *A. hyacinthus* from five localities of Karimunjawa Archipelago are in the high category of genetic diversity. However, the five populations showed a close genetic relationship of kinship. This is likely due to the small size of the population and few numbers of samples that may not represent the population. The results may aid managers of the park in the selection of appropriate propagules sources which can help to restore important data for conservation and sustain coral reef resources.

1. Introduction

Karimunjawa waters are one of the earliest marine parks in Indonesia. The park lies in the Java Sea, approximately ± 45 nautical miles from Jepara, on the north coast of Central Java, Indonesia. The park was designated as a Strict Natural Reserve on 9 April 1986 by Minister of Forestry under decree (PHKA No. 123/Kpts-II/1986) as an effort to conserve marine resources from destructive fishing activities. In 2001, all marine waters of Karimunjawa National Park (KNP) were designated as a marine conservation area by the Ministry of Forestry Decree No. 74/Kpts-II/2001 [1]. The park consists of 27 big and small islands, seven of which are inhabited. Coral communities are found vigorously to a depth about 15 m with Pocillopora and Acroporidae being the dominant family. According to survey conducted by Wildlife Conservation Society (WCS), there are 63 genera of 15 families of scleractinian corals. Recent trends in the average of coral cover were moderate (around 40–60 %) [2] with similarity index between 0.43 to 0.91 [3].

KNP represents well the population-related reef management issues that are common in developing countries [4], particularly in the Indo-Pacific where efforts to mitigate local impacts that reduce reef

resilience are currently scarce [5]. With most populated island in Indonesia is nearby (Java Island, > 60 % of total nation population), KNP pose a significant challenge for reef managers to address issues of balancing demands for reef-generated resources with conservation efforts [6]. Though it was reported that co-management frameworks as adaptive measures to gain trust, collaboration and a sense of ownership by reef users, improved some ecological condition of the reefs [1], however, extensive human activity related to tourism [7], coupled with emergence of coral disease [8, 9, 10], coral bleaching [11, 12] and increasing local demands of coral reefs resources by a growing local population [13] can contribute to reefs's decline condition.

For coral reefs, potential adaptation ability to re-establish after disturbances is important. There are various factors that may facilitate local adaptation including genetic factors. Understanding coral diversity is essential in managing coral reefs ecosystem especially when many of the reefs are in degraded condition [14]. When MPA network is designated, it is important to understand genetic connectivity among reefs and which areas are particularly rich in species to ensure that the designated protected areas are self-sustaining and possibly export propagules to neighboring reefs [15]. Most corals release gametes in a great event namely mass corals spawning [16] which were then dispersed by ocean currents into various distances from the natal reefs. Knowledge of genetic diversity is a need to understand the coral distribution and the source of coral larvae which is important to replenish coral reefs ecosystem. However, genetic diversity has rarely been corporate in designing a Marine Protected Area [14].

DNA barcoding (DBC) is an alternative to traditional taxonomic methods that could become a useful tool for coral reef conservation [17]. Cytochrome c oxidase subunit 1 (COI) has been proposed as the main barcoding gene for metazoans [18]. DNA barcoding has been extensively used to discriminate between closely related species, to identify new, cryptic or invasive species, and to assess biodiversity across many animal phyla [19]. The effectiveness of this approach clearly depends on the availability of extensive databases of COI-5 barcode sequence standards [17]. Based on the results of DNA barcode of 90 species of corals from 44 genera belonging to 14 families, suggested that the criterion was not being appropriate for most scleractinian corals (or anthozoans in general) because this region evolves very slowly in these organisms and consequently both inter- and intra-specific variation are extremely low [20]. However, taxonomy of *Stylophora pistillata* was successfully demonstrated comprises of four deeply divergent clades based on COI divergence and phylogeny of the coral [21].

Genus Acropora, are by far the most diverse living reef-building coral genus, with 114 species recognized worldwide [22] and others possibly unnamed. The genus distributes on most tropical reefs throughout the world, except the eastern Pacific Reefs [23]. Acropora is distinguished from all other extant coral genera by a unique form of polyp structural. Corallites are small, divide into two types axial and radial. Corallites have septa in two cycles or less and columellae are seldom developed [24]. Acropora plays a dominant role in the species composition and abundance of many Indonesian reefs [25]. Acroporid is a good example of studying reefs connectivity since they have the most genetic information available for any coral genus worldwide. Acroporids also knew to have largest geographical ranges compare to other corals family [26].

Here, we present a comprehensive analysis of intra- and interspecific COI variabilities in *Acropora hyacinthus* to determine connectivity patterns and genetic diversity of the coral on Karimunjawa Archipelago reefs. The results may aid managers of the park in the selection of appropriate propagules sources which can help to restore important data for future coral reef conservation and sustain coral reef resources.

2. Materials and Methods

2.1. Sample collections

Sampling was carried out at Karimunjawa Archipelago (KNP) at five study sites namely Seruni, Genting, Sambangan, Menjangan Kecil and Cilik Island. One site each were established at three

islands, in approximately 2–7 m depth. Sampling trip was carried out in May 2014 and June 2015. Snorkeling or scuba was used to sample > 20 individuals of *A. hyacinthus* per reef, a sample size adequate for genetic diversity tests [27].

Each coral colony as a sample was documented for identification. The samples from branches were cut for 1–2 cm of random colony using cutting pliers. At least 1 m distanced from one colony to another colony to avoid collected clone colony. As branches colony, *A. hyacinthus* can reproduce through fragmentation [28]. Small pieces were then put into a 2 mL labeled cryovial. To avoid re-sampled, each colony that was already cut was tagged using the small plastic number and cable ties. Table 1 has specific site information along with the number of corals collected at each site. Samples were preserved in 95 % ethanol until used. In total, 104 coral branches were obtained from KNP.

Table 1. Sample locations and number of collected colonies.

Location	Ordinate of sample locations	Number of colonies
Genting Is.	E 110º35'11.6" S 05º50'38.8"	24
Seruni Is.	E 110º26'24.0" S 05º53'02.7"	20
Sambangan Is.	E 110º34'57.8" S 05º51'15.0"	20
Menjangan Kecil Is.	E 110° 24' 22,3" S 05° 53' 23,3"	20
Cilik Is.	E 110° 30' 24,7" S 05° 49' 18,6"	20
Total		104

2.2. DNA Extraction

A quick and simple DNA extraction method with the resin, Chelex 100 (BioRad) was done. A 0.2 mm section of tissues were scrapped using sterile spatula and place into 1.5 mL tubes filled with 300 uL 10 % Chelex. The samples were then vortexed in Chelex slurry for 10–15 seconds briefly at high speed in a microcentrifuge, incubated for 20 minutes at 95 °C and vortexed again for 10–15 seconds to ensure that all contents are in the bottom of the microcentrifuge tube. The supernatant was directly used for PCR amplification. DNA concentration was quantified using Nanodrop with spectral length on $260 \cdot 280\ \mu m^{-1}$ and $260 \cdot 230\ \mu m^{-1}$.

2.3. PCR Amplification

Amplifications were performed in 25 µl volume master mix PCR solution. Universal primer for COI gene, consisted of forward primer LCOI1490: 5'-CAAATCATAAAGATATTGG-3' and reverse primer HCOI2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [29] were used for amplification. Master mix PCR solution containing 1 µl of each primer, 1 µl of DNA Template (< 250 $ng \cdot \mu l^{-1}$), 12.5 µl of GoTaq ® Green Master Mix (Promega, USA) and 9.5 µl of Nuclease-Free Water (Promega, USA). Thermal cycle condition was following protocol by [20]. PCR products were analyzed using 1 % agarose gel. PCR products that have DNA band were directly sequenced at 1st Base Laboratory using both of primers. Sequencing results were analyzed using MEGA 6.0 for alignment process and Phylogenetic tree was constructed using Phylogenetic Analysis Using Parsimony (PAUP) software [30].

3. Results and Discussion

In total, there were 32 sequences resulted from Menjangan Kecil Is., Cilik Is., Genting Is., Seruni Is., and Sambangan Is., Karimunjawa Archipelago, Java Sea. The resulting Oxidase I (CO I) of the mitochondrial genome DNA (mtDNA) sequences were corresponding to the genotype that was analyzed for homologies of sequences in the database using BLAST searches. BLAST analysis showed that all 32 sequences were closely related to *Acropora hyacinthus* under 99 % similarity. All nucleotide sequences have been deposited in the GeneBank database under accession number LC189268–LC189316.

3.1. Phylogenetic Tree

Reconstruction of the phylogenetic tree was done using PAUP* 4.0 (Phylogenetic Analysis Using Parsimony) [30] with parsimony method and 1,000 times boots trap. Kinship relationship was observed using TreeView program.

Phylogenetic construction based on COI sequences found 6 distinct clusters of *A. hyacinthus*. Outgroup using DNA data base of *Montipora* sp. Phylogenetic tree of *A. hyacinthus* was shown in figure 1.

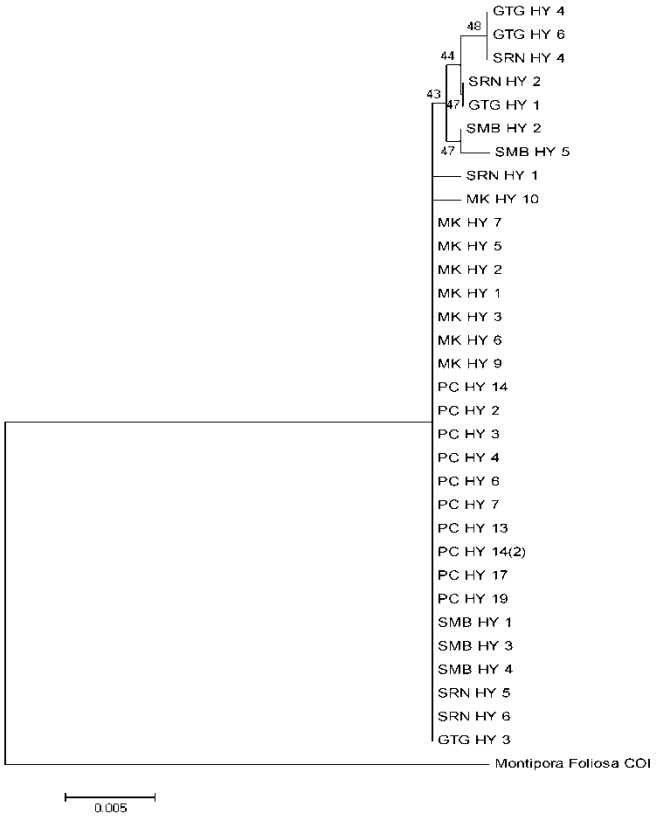

Figure 1. Phylogenetic tree construction of *Acropora hyacinthus* collected from Menjangan Kecil Is. (MK), Cilik Is. (PC), Genting Is. (GTG), Seruni Is. (SRN) and Sambangan Is. (SMB), Karimunjawa Archipelago, Java Sea. There 6 different clades observed from the tree. *Montipora foliosa* was used as outgroup.

Clade 1 was comprised of three samples of Genting and Seruni Isl. (GTG HY4, GTG HY 6 and SRN HY 4), clade 2 consisted each colony of SRN HY 2 and GTG HY 1, clade 3 consisted only two samples from Sambangan Isl. (SMB HY 2 and SMB HY 5), while clade 4 (SRN HY 1) and 5 (MK HY 10) consist only one sample each. Clade 6 is the biggest clade that comprised of mix samples collected from five different reefs (Menjangan Kecil, Cilik, Genting, Seruni and Sambangan island) (MK HY 7, MK HY 5, MK HY 2, MK HY 1, MK HY 3, MK HY 6, MK HY 9; PC HY 14, PC HY 2, PC HY3, PC HY 4, PC HY 6, PC HY 7, PC HY 13, PC HY 14(2), PC HY 17, PC HY 19; SMB HY 1, SMB HY 3, SMB HY 4, SRN HY 5, SRN HY 6 and GTG HY 3) (figure 2).

When comparison was made with *A. hyacinthus* population from Raja Ampat, a similar result was obtained. *A. hyacinthus* of Raja Ampat has grouped into 6 different clades [31]. Samples of *A. hyacinthus* from Kofiau District was collected from Deer Island and Boo Island that was separated by more than 100 km, while samples from Karimunjawa Archipelago was collected from two different

cluster islands namely Seruni, Sambangan and Genting cluster and Cilik and Menjangan Kecil cluster islands that separate by most far 60 km only. However, the Menjangan Kecil Island and Cilik Island were set behind the main Karimunjawa Island that faces the open sea while Seruni, Sambangan and Genting were set inside lagoonal area of Karimunjawa main island.

3.2. Analysis of Genetic Diversity

Genetic diversity of *A. hyacinthus* was observed using DNAsp [32]. Based on statistic analysis of genetic diversity of 32 samples showed 15 haplotypes (table 2). There were 11 haplotypes (73.3 % of total samples) are unique haplotypes that consisted only one sample each. While four haplotypes (26.7 %) are more common haplotypes with haplotype 1 as the highest member of samples (13 samples). Estimation of haplotype diversity value of *A. hyacinthus* was 0.8286 which categorized has a high genetic diversity [33]. It is likely that population of *A. hyacinthus* at Karimunjawa Archipelago showed high genetic diversity.

Table 2. Haplotype diversity distribution of *Acropora hyacinthus* from Karimunjawa Archipelago, Java Sea; HY, *Acropora hyacinthus*; MK, Menjangan Kecil Is.; PC, Cilik Is.; GTG, Genting Is.; SRN, Seruni Is.; SMB, Sambangan Is.

No	Haplotype	Number of samples	Samples code
1.	Haplotype 1	13	MK HY 1, MK HY 5, MK HY 6, MK HY 7, MK HY 9, PC HY 14, PC HY 6, PC HY 7, PC HY 13, PC HY 14(2), PC HY 17, PC HY 19, GTG HY 3
2.	Haplotype 2	3	MK HY 2, SRN HY 5, SRN HY 6
3.	Haplotype 3	1	MK HY 3
4.	Haplotype 4	1	MK HY 10
5.	Haplotype 5	3	PC HY 2, PC HY 3, PC HY 4
6.	Haplotype 6	1	SMB HY 1
7.	Haplotype 7	1	SMB HY 2
8.	Haplotype 8	1	SMB HY 3
9.	Haplotype 9	1	SMB HY 4
10.	Haplotype 10	1	SMB HY 5
11.	Haplotype 11	1	SRN HY 1
12.	Haplotype 12	2	SRN HY 2, GTG HY 1
13.	Haplotype 13	1	SRN HY 4
14.	Haplotype 14	1	GTG HY 4
15.	Haplotype 15	1	GTG HY 6
	Total	32	

Genetic distance within island showed that samples collected from Seruni Isl. has farthest genetic distance (0.003 ± 0.003) followed by samples from Sambangan (0.002 ± 0.001) and Genting Isl. (0.002 ± 0.003). While samples from Menjangan Kecil and Cilik Isl. Showed nearest genetic distance. When comparison was made between a distance of different reefs, the closest genetic distance was shown by samples from Menjangan Kecil and Cilik Isl. (0.000) (table 3).

Table 3. Genetic distance within distance of *Acropora hyacinthus* from Karimunjawa Archipelago; d, distance; S.E., standard error.

Island name	D	S.E.
Menjangan Kecil	0.000	0.000
Cilik	0.000	0.000
Sambangan	0.002	0.001
Seruni	0.003	0.003
Genting	0.002	0.003

Distributions of species of the coral genus Acropora in the Indonesian archipelago show a duality reminiscent of the Wallace's Line patterns seen in terrestrial animals and plants, rather than the concentric pattern predicted by the "center of origin" model [34]. This duality is due to an overlap of Indian Ocean species distributions diminishing eastwards and Pacific Ocean species distributions diminishing westwards within the archipelago [34]. Additionally, a large number of species with broad Indo-Pacific distribution, as well as some regional endemics, occur within the archipelago. Thus although Indonesia has a high species number overall, this is due to the presence of a composite fauna with strong regional differences.

Using multi-locus nucleotide sequence data, [26] recently described two, previously unsuspected, cryptic species complexes within the *Acropora syngameon* (i.e., groups of species connected through a genetic exchange). Acropora is categorized as the most diverse and abundant genera of reef-building corals [22, 24]. The genus is notorious for high levels of shared polymorphism between species, which is due, in part at least, to widespread genetic exchange through introgression [35, 36, 37]. Despite the absence of any fixed sequence differences, utilized allele frequency variation at 10 loci to demonstrate that two common coral species in Australia, *A. cytherea* and *A. hyacinthus*, actually represent species complexes minimum two and four cryptic species. These cryptic species were discovered in complete sympatry and form part of an extended syngameon with a complex network of gene flow among species. Cryptic species were identified by the 'genotypic cluster' species definition, which defines species as distinguishable genotypic groups.

The results of this research indicate that corals *A. hyacinthus* from Karimunjawa Island is categorized as high of genetic diversity, though they showed a relatively close genetic relationship of kinship. We need run a second gene such as microsatellite or intron sequencing to confirm possibility that population of *A. hyacinthus* at Karimunjawa Archipelago may have a cryptic variation as suggested by [26]. However, the possibility that population of *A. hyacinthus* at Karimunjawa Archipelago has high diversity could not be excluded. Therefore, managers of the KNP need more appropriate approach to resources include the genetic diversity.

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