Diversity and Distribution of Euphyllidae Corals in Tioman Island: Emphasis on the Genetic Variation of *Euphyllia cristata*

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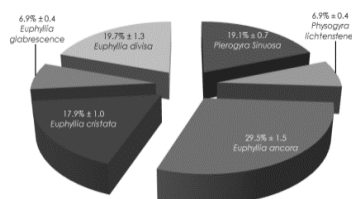[c]Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia

Graphical abstract

Abstract

Euphyllidae corals are among the most beautiful corals that have been exploited for the aquarium trade all over the world. In Malaysia, the information on these coral species particularly on the diversity and genetic variation is not fully documented. Realizing to this matter, the diversity and distribution of Euphylllidae corals were investigated at 13 sampling stations in Tioman Island. The diversity and distribution of Euphyllidae corals were observed and recorded using the Coral Video Transect (CVT) method. The captured images were analyzed using Coral Point Count with Excel extension (CPCe) software. The samples of *Euphyllia cristata* were collected at three different zones in Tioman Island (East Coast, West Coast and Isolated Island). The samples were extracted and mitochondrial Cytochrome Oxidase 1 gene primer was used to study their genetic variation. A total of six species belong to three genera of *Euphyllia*, *Plerogyra* and *Physogyra* were recorded. Benuang Bay (East Coast) and Bayan Bay (Isolated Island) have shown higher diversity and distribution of Euphyllidae corals among the sampling stations. The Shannon-Weinner index (H') of Benuang Bay and Bayan Bay were 1.42 and 1.43 respectively. The West Coast zone had the lowest diversity and distribution of Euphyllidae corals due to massive coastal development and loads of human intervention. The genetic sequences from the East Coast and West Coast were closely related. The pairwise distance between these two zones is 0.003. There is a slight difference in the sequence from the Isolated Island. The pairwise distance of West Coast-Isolated and East Coast-Isolated are 0.021 and 0.025 respectively. However, it is relatively low to consider there is evolutionary divergence between the samples. Thus, no genetic variation was observed between all *Euphyllia cristata* taken at three different zones of Tioman Island. This study has shown that there was a highly significant difference (p<0.01) in the diversity and distribution of Euphyllidae corals with respect to sampling stations.

Keywords: Euphyllidae corals, diversity, distribution, CVT method, DNA analysis

1.0 INTRODUCTION

Euphyllidae corals are among the most beautiful of all scleractinian corals in the ocean world [1]. These Euphyllids are usually colourful with large protuberant polyps and massive colonies, making them attractive in the reef areas [1, 2]. They mostly inhabit sheltered areas such as shallow and protected tropical reef slopes environments as they prefer areas with the limited current and wave action [3]. Most of the Euphyllids are commonly found in reef slope where the wave action is mild with depth ranging from 9 to 15m [4].

Euphyllidae corals composed entirely with five zooxanthellate genera namely as *Euphyllia, Catalaphyllia, Plerogyra Physogyra* and *Nemenzophyllia* [1]. All of these species are known to be commercially targeted for the aquarium trade due to their attractive appearances and aesthetic value [3]. Based on the International Union for Conservation Nature and Natural Resources (IUCN) Red List agency, most of the Euphyllids have been categorized under threatened due to over-exploitation for aquarium trade. More than a thousand of scleractinian corals which included Euphyllidae corals have been exported throughout the world annually [5].

In 2005, a total of 28,000 live corals of *Euphyllia sp.* have been exported by Indonesia where the live coral specimens were collected from the central of Indo-Pacific, the central of Indian Ocean, and the Oceanic West Pacific [3]. Recent study indicated that a nearly of 20% of *Euphyllia sp.* were transported in a three years period starting from 2009 to 2011 with Europe as the major consumer and Indonesia as the major supplier [6]. Apart of being over-exploited for marine aquarium trade, the environmental stress such as global warming, ocean acidification and coral disease have reduced the numbers of these coral species [7, 8, 9, 10].

In Malaysia, the information about the diversity and distribution of Euphyllidae corals is not well documented. This lack of informations inhibit effective decision making concerning coral reefs particularly on these species. Therefore, this study is considered important to be carried out. As a first attempt, Tioman Island has been chosen as a study site due to the abundances of coral species among other islands in the East Coast of Peninsular Malaysia. This study aims to determine the diversity and distribution of Euphyllidae corals with emphasis on the genetic variation of *Euphyllia cristata.*

2.0 METHODOLOGY

2.1 Study Area

A total of 13 sampling stations were selected in this study. All the sampling stations were divided into three zones: East Coast zone (EC) (Benuang Bay, Benuang and Dalam Bay), West Coast zone (WC)

(Tomok Island, Genting Village, Renggis Island, Soyak Island and Terdau Bay), and the Isolated Island (II) (Gado Bay, Bayan Bay, Tulai Bay, Sepoi Island and Labas Island) as shown in Figure 1. The sampling stations were chosen based on the difference in environmental settings, coastal developments and human activities. All the reef sites were categorized as fringing reef areas with a depth range from 3m to 16m. The good tourism facilities and infrastructure has attracted many local and international tourists to visit this place.

2.2 Corals Survey Method

The survey was carried out for three months started from July to September 2013. The Coral Video Transect (CVT) method used in this study was adapted from the technique developed by the Australian Institute of Marine Science (AIMS) with some modification. Four 30m transect lines were placed in parallel form with 2m interval for each transect. A total of 52 transect lines were used in this study. The underwater video camera was used to record the benthic communities along the transect lines with distance of the camera is approximately 20 to 25cm from the substrate and at a perpendicular angle to the bottom. The camera was ran along the transect lines at a speed of six minutes per transect line to get clear and sharp images for laboratory analysis. Additional corallites close-up pictures were also taken for the identification process.
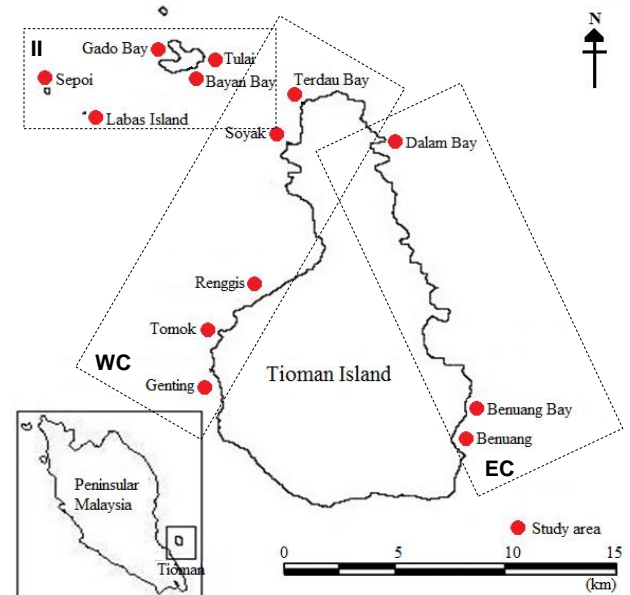
Figure 1 Location of thirteen study areas in Tioman Island, Dalam Bay (02°52'28.30"N 104°11'16.98"E), Benuang Bay (02°45'35.65"N 104°13'19.67"E), Benuang (02°44'37.85"N 104°12'58.35"E), Genting Village (02°45'57.34"N 104°07'06.97"E), Tomok Island (02°47'31.61"N 104°07'18.95"E), Renggis Island (02°48'36.58"N 104°08'09.41"E), Soyak Island (02°52'29.72"N 104°08'49.89"E), Terdau Bay (02°53'20.01"N 104°09'16.61"E), Gado Bay (02°54'53.4"N 104°06'31.4"E), Bayan Bay(02°54'35.64"N 104°06'42.36"E), Tulai Bay (02°54'49.9"N 104°06'50.3"E), Sepoi Island (02°53'55.31"N 104°02'58.33"E), Labas Island (02°53'19.06"N 104°03'58.06"E).

2.3 Video Processing & Data Analysis

Coral Point Counting with excel extension (CPCe) software was used to analyzed the images extracted from the underwater video recorded. All the relevant data and types of corals intercepted of each picture were identified based on Coral Finder toolkit and reference book of Corals of the World. Data recorded was analyzed to get percent coverage of Euphyllidae corals for all sampling stations. In addition, Shannon- Wiener Index was applied to calculate the diversity index of corals for each sampling station. Chi-square test was conducted to see any significance differences between sampling stations and coral genera using software of SPSS version 20.

2.4 Molecular Studies on the DNA of *Euphyllia cristata*

Samples of *Euphyllia cristata* were collected by cutting 1 to 2cm fragment from individual coral colony. Tissue and skeletal material were stored in 4°C ice container. Total genomic DNA of *Euphyllia cristata* were extracted using QIAamp DNA mini kit. The extraction processes were based on GIAquick Gel Extraction Kit Protocol. Primer of Cs-F18 (5'-GGACACAAGAGCATATTTTACTG) and Cs-R18 (5'-CTACTTACGGAATCTCGTTTGA) were designed from the published data of 74 universal primers for characterizing the complete mitochondrial genomes of sclerectinian corals and were used to amplify [11]. Polymerase chain reaction (PCR) was conducted using Promega GoTaq® DNA polymerase with buffers provided. Thermocycler reaction parameters were: initial denaturing step at 98°C for 3 min, followed by total 30 cycles of 98°C for 0.3 min, 55°C for 0.5 min, and 72°C for 0.5 min. A final extension step at 72°C for 10 min to complete the reaction. PCR amplifications were confirmed by running products on 1% agarose gels. UV transilluminator (Alphaimager™ 2200, Germany) was used to visualize the products bands. All successful amplifications were directly sequenced (outsourced to sequencing service at 1st BASE Sdn Bhd, Malaysia).

2.5 Sequence Data Analysis

All the sequences were aligned using Clustal X2 for multiple sequence alignment. Sequence of Cytochrome Oxidase 1 of *Euphyllia ancora* was used as outgroup. Pairwise distance bootstrap Neighbour-joining and Maximum Parsimony phylogenetic trees were generated using MEGA version 4 as for the analysis of the genetic variation of *Euphyllia cristata*.

3.0 RESULT AND DISCUSSION

3.1 Distribution and Evenness Index of Euphyllidae corals

In Tioman Island, a total of six species belong to three Euphyllidae corals genera were identified. Based on the average percent coverage of Euphyllidae corals (Figure 2), species of *Euphyllia ancora* and *Euphyllia divisa* were found abundances among others with 29.5% and 19.7% coverage respectively. The least abundance of species were *Physogyra lichtenstenei* and *Euphyllia glabrescens* with only 6.9% average coverage. Based on Table 1, Euphyllidae corals were found in almost all sampling stations except for Genting Village. Benuang Bay and Bayan Bay have the highest abundance of Euphyllidae corals among the sampling stations. Shannon-Weiner diversity index (H') and Evenness index (EH') of Euphyllidae corals at all sampling stations is shown in Table 2. Benuang Bay and Tulai Bay have shown higher value of diversity index (H') with 1.43 and 1.42 respectively compared to other sampling stations. Benuang Bay also represented the highest value of evenness index (EH') with 2.25 among the other sampling stations. Tomok Island and Genting Village have shown zero diversity index (H') and Evenness index (EH') due to the less than one species present in the areas. A total diversity index (H') and Evenness index (EH') of Euphyllidae corals found in Tioman Island was 1.67 and 0.93 respectively. However, detail of Chi-square analysis of Euphyllidae corals with respect to 13 sampling stations has shown high significant difference (p<0.01).
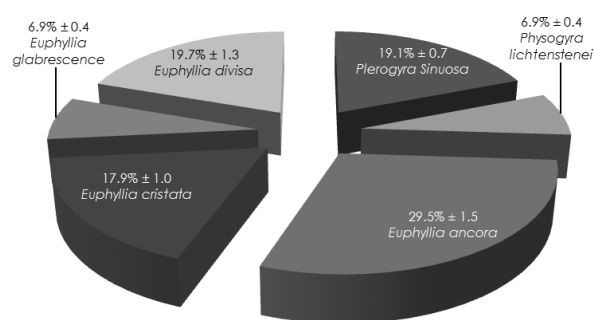

Figure 2 Average Percent coverage of Euphyllidae c orals in Tioman Island

The variation of diversity and distribution of these corals are mainly due to their different ability to adapt with certain environmental settings and anthropogenic disturbances [12]. From the result, the west coast zone has significantly less diverse of Euphyllidae corals especially in Tomok Island, Genting Village and Renggis Island.

Table 1 Diversity and distribution of Euphyllidae corals in 13 sampling stations, Tioman Island

No.	Euphyllidae coral	Sampling Station												
		East Coast Zone			West Coast Zone					Isolated Island				
		Benuang Bay	Benuang	Dalam Bay	Tomok Island	Genting Village	Renggis Island	Soyak Island	Terdau Bay	Gado Bay	Bayan Bay	Tulai Bay	Sepoi Island	Labas Island
	Plerogyra													
1	*Plerogyra sinuosa*	+	-	-	+	-	+	+	+	+	-	+	+	+
	Physogyra													
2	*Physogyra lichtenstenei*	+	-	-	-	-	-	+	-	+	-	+	+	+
	Euphyllia													
3	*Euphyllia ancora*	++	+	-	-	-	-	-	+	+	-	++	+	+
4	*Euphyllia cristata*	++	+	+	-	-	-	-	+	-	+	+	-	+
5	*Euphyllia glabrescence*	-	+	-	-	-	-	+	-	-	+	+	-	+
6	*Euphyllia divisa*	++	+	+	-	-	-	-	+	+	-	+	-	-

+ = 1-10   ++ = 11-20   +++ = 21-30 ++++ = 31-40   +++++ = >40

Table 2 Shannon-Weiner diversity index (H') and Evenness index (EH') of Euphyllidae coral in all sampling stations, Tioman Island

Location		No. of Species (S)	Total of Species (∑)	Diversity Index (H')	Evenness Index (EH')
East Coast Zone	Benuang Bay	5	47	1.42	2.25
	Benuang	4	13	1.38	1.00
	Dalam Bay	2	13	0.69	1.00
West Coast Zone	Tomok Island	1	2	0	0
	Genting Village	0	0	0	0
	Renggis Island	1	1	0	0
	Soyak Island	3	6	0.87	0.79
	Terdau Bay	4	17	1.14	0.82
Isolated Island	Gado Bay	5	13	1.35	0.84
	Bayan Bay	2	7	0.60	0.87
	Tulai Bay	6	34	1.43	0.80
	Sepoi Island	3	8	0.74	0.93
	Labas Island	5	13	1.26	0.78

These locations are heavily populated where a lot of coastal developments such as resorts, jetties and residential areas. The extensive of human activities along the coastal areas may represent the greatest threat to corals [13]. They might affect the coral population by causing stress to the corals and lead to coral mortality when they are exposed in longer period of time [14, 15]. The other activities such as trampling of corals during snorkeling and diving, and boat anchoring can cause coral partial mortality [16, 12]. At Terdau Bay, the numbers of Euphyllidae corals present is quite high compared to the other sampling locations in the West Coast zone due to less development and human disturbances.

As for the East coast zone, the number of Euphyllidae corals found at each sampling station is higher compared to the West Coast zone. The high abundance of Euphyllidae corals is due to the geographical and morphological factors of that area. As directly facing to the open sea, the expose of high wave and current especially during the northeast monsoon season have reduced the sedimentation rate thus helps in coral propagation and establishment. The bottom morphology of the area which is dominated by rocky substrates that provides sheltered areas from strong wave and currents has encouraged corals to grow. Having the resemblance condition with the East Coast zone, the Isolated Island also provides the suitable condition for Euphyllidae corals to grow [4].

## 3.2 Genetic variation of *Euphyllia cristata* in Tioman Island

The DNA sequences of *Euphyllia cristata* (EC) from three different zones were analyzed using a standard bioinformatics tools. The sequences were labeled as EC1(West Coast Zone), EC9 (Isolated Island) and EC15 (East coast zone). All the sequences were analyzed by referring to the Cytochrome Oxisdase subunit 1 gene of *Euphyllia ancora* (EACOX1), Based on the pairwise distance analysis (Table 3), it can be seen that all the three samples of EC were not similar to the sequence of EA. However, it can be seen that the value of the distance of the combination of EC1-EC15, EC1-EC9 and EC15-EC9 were 0.005, 0.014 and 0.014 respectively. It can be said that the combination of EC1-EC15 had a closer relationship compared to the other combinations. Based on Figure 3 and 4, the phylogenetic tree shows that EC1 and EC15 were in the same clade while EC9 is on a different clade. However, all the samples were down lined from the same root which is from EA

Table 3 Pairwise Distance between three samples of EC and EACOX1

	EC1FRC	EC9FRC	EC15FRC	EACOX1
EC1FRC	-			
EC9FRC	0.021	-		
EC15FRC	0.003	0.025	-	
EACOX1	1.215	1.242	1.111	-
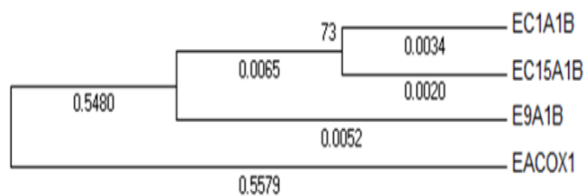
Figure 3 Neighbour-Joining phylogeny between three samples of EC and EACOX1
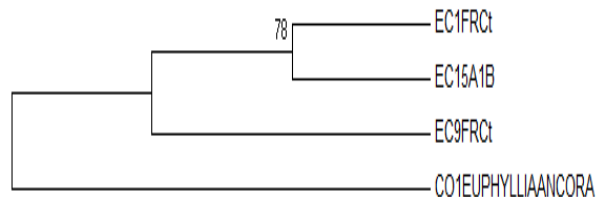
Figure 4 Maximum Parsimony phylogeny between three samples of EC and EACOX1

The result showed that EC1 (West Coast Zone) and EC15 (East Coast Zone) were closely related compared to EC9 (Isolated Island). This is due to the coastal development conditions and human activities occured in both zones. The presence of these factors might give a negative impact to the corals that lead to the genetic diversity for some marine animals to survive and adapt with the changes in their environments [17]. The genetic diversity is crucial for the survival of a species and it forms the basis of variation and population viability [17].

However, the distance differences between each zone were less than 0.1 and it is relatively low to consider there is evolutionary divergence between all the samples. A standard threshold for genetic divergence is 10X the mean intraspecific variation and more than three percent threshold can be considered there is genetic divergence [18]. A theory infers that there may be a genetic variation between the species samples taken in different sampling stations due to different environmental condition. However, the distance between all the sampling stations are too close for the species to genetically evolve. It is difficult to genetically differentiate the marine organisms because the ocean is illimitable considering few factors such as dispersal of larvae over large distances and boundary to prevent gene flow in the ocean [19].

In addition, the genetic variation among all three samples cannot be proven, as the primer used is only partial dominant sequence of *Euphyllia cristata* CO1 gene with product size of 973bp taken from GenBank. Previous study recorded the size of *Euphyllia ancora* CO1 gene is 2300bp [11]. The size of the CO1 gene between *Euphyllia cristata* and *Euphyllia ancora* can be compared because both species are categorized in the same family of Euphyllidae. Comparing the size of partial sequence and the complete dominant sequence, there is still 1327bp that is still unknown. This can be suggested that variation of the sequence may occur within the 1327bp region.

Additionally, the CO1 sequence data for coral phylogeny is unambiguously alignable and it contains no indels. This give a strong evidence that there is no genetic variation between the samples [20]. The absent of indels show there is no insertion or deletion of bases in the CO1 gene of the organisms. Thus, it shows that the CO1 gene is a conserved gene and it will not evolve or mutated even under a stressful environment due to absence of indels.

5.0 CONCLUSION

This study has shown that the East Coast and Isolated areas had better diversity and distribution of Euphyllidae corals as compared to West Coast zones. It was clear that the geographical and morphological were important factors in influencing the diversity and distribution of Euphyllidae corals. High rates of coastal developments and human activities were also affect the coral population that cause persistently elevated rates of mortality. The study on genetic variation of *Euphyllia cristata* between each sampling zones showed no evolutionary divergence between the samples. The CO1 gene used for phylogeny test is a conserved

region that is evolve unusually slow. Genetic variation also is hardly occur between small distances of locations. Thus, no evolutionary divergence can be proven between samples taken.

6.0 RECOMMENDATIONS

Further studies on the diversity and distribution of endangered coral species should be done in longer period of time since there are only a few references available regarding this matter. This is to ensure that the coral species available in Malaysian waters would not extinct. The Department of Marine Park Malaysia should take action if there is any exploitation of coral species. Besides that, the influence of human activities such as snorkelers and divers should be considered into accounts. Inexperienced divers and snorkelers may tend to trample on the coral and cause coral to break. As a matter of facts, the Department of Marine Park Malaysia should monitor this activity since snorkeling and diving mostly done within protected areas (2 nautical miles). Carrying capacity in terms of snorkelers and divers should also be applied by resort operators under administrative of the Department of Marine Park Malaysia. In depths study, study on genetic variation of this species can be improvised by comparing *Euphyllia cristata* in East Coast of Peninsular Malaysia with *Euphyllia cristata* in West Coast of Peninsular Malaysia. Large spatial distances may have evolutionary divergence due to different geographical area. Apart from that, the genetic study on this species also can be further improved by using a complete dominant sequence of CO1 gene to examine the genetic variation for complete sequence.

Acknowledgements

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