



Close genetic connectivity of soft coral *Sarcophyton trocheliophorum* in Indonesia and its implication for marine protected area

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ABSTRACT

The genetic connectivity of soft coral is influenced by current and distance between islands. The complexity of islands and geographical region in Indonesia might influence the distribution of soft corals. The information of genetic connectivity can be used to design marine protected areas and to avoid destruction and possible extinction. The objective of the present study was to analyze genetic connectivity of one species of soft coral, *Sarcophyton trocheliophorum*, in three populations spanning Java, Nusa Tenggara, and Sulawesi's waters, and to describe its implication for marine protected area. The mitochondrial protein-coding gene (750 bp of ND2) was used to analyze genetic population structure and genetic connectivity. Genetic connectivity was found in all populations with F_{st} value of 0.227 to 0.558, indicating populations had the close genetic relationship. The local and Indonesian currents were expected to distribute the larva to islands as a stepping stone, they moved slowly to spread them self far away. Tanakeke island (Sulawesi population) might be a center connectivity of *S. trocheliophorum* populations. This island connected with islands in west and east Indonesia, therefore that area need to protect.

Keywords: Connectivity, Conservation, Marine Protected Area, *Sarcophyton trocheliophorum*, Soft coral

INTRODUCTION

The distribution patterns of marine organisms can be detected through their genetic connectivity (Madduppa *et al.*, 2014a). The spread of organisms is influenced by several factors such as currents, the biology of larvae, and geographical of location (Adam, 2014). The close and complex distance between islands in Indonesia serves as a stepping stone to facilitate the dispersal of coral larvae. In addition to that, the strong pattern of currents in Indonesia also assists in the process of larval dispersal (Salim, 2012). Current transportation is an important tool in the process of distribution of sessile animal larvae (Benayahu and Loya, 1986).

Sarcophyton trocheliophorum is one of the members than compiler up the community of coral (Dai, 1993), Genus of *Sarcophyton* are one types of soft coral that found in Indonesia (Manuputty, 1996). They have two types of reproduction, sexual by gonochoric and asexual by fragmentation (Hellstrom *et al.*, 2010). The fertilization of sexual reproduction was externally (Benayahu and Loya, 1986), this way could distribute the larvae. The pelagic larvae duration of *Sarcophyton sp.* is about 14 days (Benayahu and Loya 1986). On coral reefs community, *S. trocheliophorum* become one of the organisms that contributes to primary productivity in the sea (Hoytema, 2016). *S. trocheliophorum* lives symbiont with zooxanthellae, they could produce an oxygen and organic matter. This species is also included in the marine ornamental trade which has a high economic value (Green and Shirley 1999). However, the high demand for soft corals is not balanced with the presence in the natural coral reef ecosystem. Human activity has caused degradation of coral reef ecosystems (Spalding *et al.*, 2001). Coral reef ecosystems have been degraded by 10-50% over the last 50 years (Wilkinson, 2002). This rapid exploitation might give an impact on the distribution of the species naturally.

Human activities and the impact of global warming may accelerate the damage of coral reef ecosystems and their associated organisms (Madduppa *et al.*, 2013). It will be impacted on the local or mass extinctions (Madduppa *et al.*, 2014b). Indonesia has the highest extinction rate than any other country in the world (Brooks, 2002). This is evidenced by many local species being classified on the Red List of extinction and danger. On the other hand, the mass extinctions will impact the structure of the species in the ecosystem, thus decreasing the organism's resistance to environmental change. The improvement of environment damage could use the genetic connectivity.

Genetic Connectivity is the key to conservation because it plays an important role in maintaining the population and restoring the damage of marine environment (Luque *et al.*, 2012). The connectivity patterns of Sessile animals is strongly influenced by the reproduction and dispersal of larvae (Jackson 1986). The pelagic larval stage is the early stages of life to regenerate, so it can defend its population from extinction. Furthermore, the patterns of genetic connectivity could help the management of marine protected area's (Ramadhani *et al.*, 2015), then, in turn, would preserve the biodiversity. Genetic connectivity between reefs is often overlooked when designing an area, but it is very important to ensure the continuity of the population or the protection of biodiversity (Wijayanti, 2009).

Molecular approaches have been used to reveal species identification (Kusuma *et al.*, 2016; Akbar *et al.*, 2014; Jefri *et al.*, 2015; Negara *et al.*, 2016; Prehadi *et al.*, 2015), and dynamics of populations (Madduppa *et al.*, 2014a; Saleky *et al.*, 2016; Sembiring *et al.*, 2015). Some soft coral genetic research has been done a molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. In addition, Phylogenetic relationships within the tropical soft coral Sarcophyton genera and Lobophytum (Anthozoa, Octocorallia) were done by McFadden (2006). In Indonesia, research of soft coral just about introducing biology and distribution aspects of soft coral (Manuputty, 2010; Manuputty, 2008; Manuputty 2002; Manuputty 2002). The genetic diversity of *S. trocheliophorum* were done by Kusuma *et al.*, (2016), While the research on genetic connectivity of soft coral on Indonesia has never been done. Therefore, the aim of this study was to analyze the genetic population structure and genetic connectivity of *S. trocheliophorum* on Java, Sulawesi and Nusa Tenggara waters and the implications for marine protected areas in Indonesia.

MATERIALS AND METHODS

Sample Collection

Purposive random sampling was used to collect the samples in this research. 39 Samples of *S. trocheliophorum* were collected from Java waters (Tunda Island and Seribu Island), Nusa Tenggara Waters (Labuan Bajo Island and Gili Tapan Island), and Sulawesi waters (Sembilan Island, Tanah Keke island, and Dutungan Island) (Figure 1). Molecular analysis was done in Marine Biosystematics and Biodiversity Laboratory, Bogor Agricultural University, Indonesia. SCUBA diving was used to take all samples from 5 -7 m. Three cm of tissue was taken from samples, and all samples were rinsed using distilled water to remove all *zooxanthellae* that symbiont on the tissue of *S. trocheliophorum*. All samples were preserved in 96% ethanol.

Molecular Characteristic Analysis

The tissue of *S. trocheliophorum* was extracted using Qiagen extraction kit. The first step of DNA extraction was to rinse the samples using distilled water to fade the ethanol. Furthermore, the samples were refined using micromesh to make lysis of the tissue easier. The refined samples were reacted using reagent from Qiagen and incubated at 56 °C until the tissue shattered. The separation of DNA was carried out using a Centrifuge type perfect spin 24 plus at 13.000 rpm.

ND2 mitochondrial amplification was performed using PCR primers 16S647F: 5'-ACACAGCTCGGTTTCTATCTACCA-3' and ND21418R: 5'-ACATC GGGAGCCCACATA-3' (Sanchez *et al.*, 2003). PCR reactions were formulated by 3 µl DNA, 12,5 µl Q5 *high fidelity master mix*, 7 µl ddH₂O, and 1,25 µl forward and reverse primer. The PCR programs were done, pre-denaturation at 98 °C for 30 sec, denaturation at 98 °C for 30 sec, annealing at 57 °C for 30 sec, extension at 72 °C for 30 sec and final extension at 72 °C for 2 min on 35 cycles.

The electrophoresis process used 1,5 % agarose, 4 µl Ethidium Bromide, 100 mL TAE 1X, and 4 µl PCR Products which were added with 1 µl loading dye. The electrophoresis was done at 100 V for 25 min. UV transilluminator was used to visualize the bands of the products. The PCR products which had good DNA (750-800 bp) sent for sequencing.

Data Analysis

The sequences of the sample were alignment using MEGA 5.05 (*Molecular Evolutionary Genetic Analysis*) (Tamura *et al* 2011). *Clustal W* was used to edit the multiple alignments (Tamura *et al* 2011). Fixation index (Fst) was used to measure the genetic population structure (Excoffier *et al.*, 1992) by using Arlequin 3.5 (Excoffier dan Lischer 2009). The genetic distance between and within populations was assessed using Mega 5.5 (Tamura *et al.*, 2011). The genetic connectivity was analyzed using a median joining application on software Network 4.6. (<http://www.fluxus-engineering.com>).

RESULTS AND DISCUSSION

Population Structure and Genetic Connectivity

The high of genetic distance analyzed within all populations were Nusa Tenggara waters, Sulawesi waters, and Java waters, respectively (Table 1). The different topography between Gili Tapan and Labuan Bajo possibly caused the highest value of genetic distance on Sulawesi waters. Gili Tapan water is the enclosed water. Gili Tapan water has Medang Island that is located in front of Gili Tapan Water. It caused larva of *S. trocheliophorum* derived from Labuan Bajo difficulty to get into Gili Tapan or other location.

Compared to Java population, the Sulawesi population has higher genetic distance (± 1560 km). A great distance (± 600 km) of Dutungan Island, Tanakeke Island and Sembilan Island that are located on the Gulf of Bone allowed larvae of *S. trocheliophorum* difficulty to pass. Unlike other populations, population of Java had genetic distance very small. This could possibly happen because the location of Panggang Island and Tunda Island is nearby. Seasonal east and west currents also allow the exchange larvae on each season.

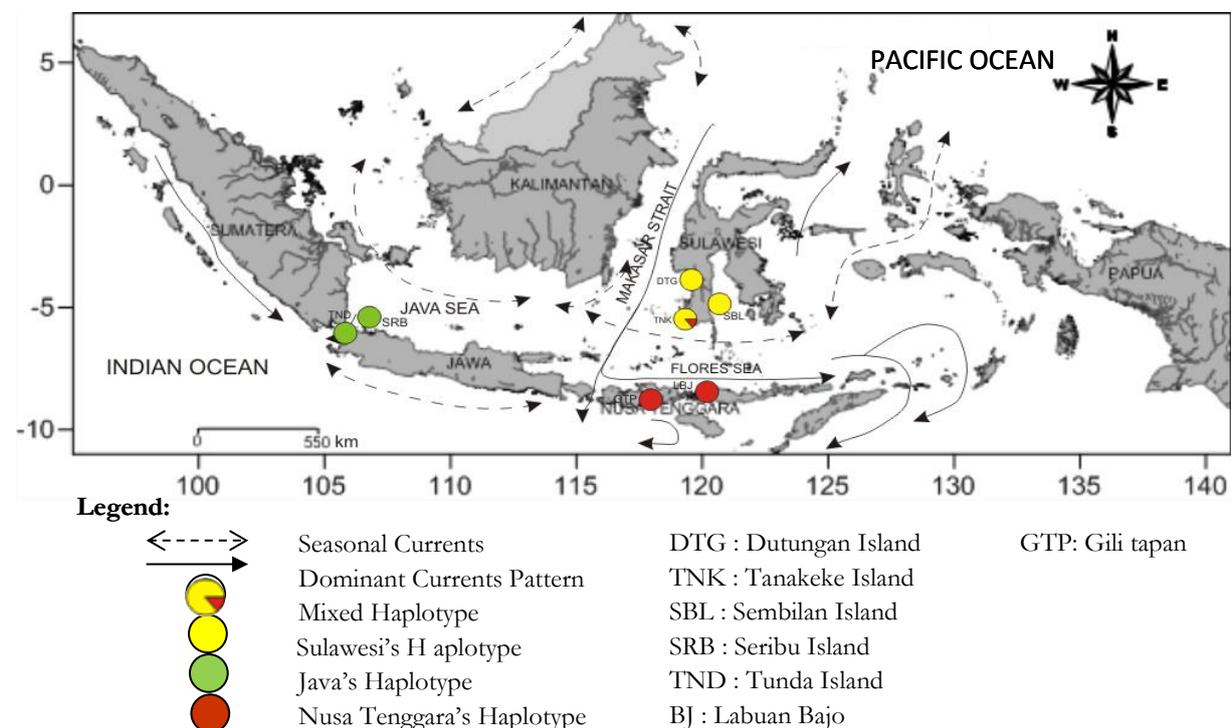


Figure 1. Haplotype distribution maps and dominant currents pattern in Indonesia

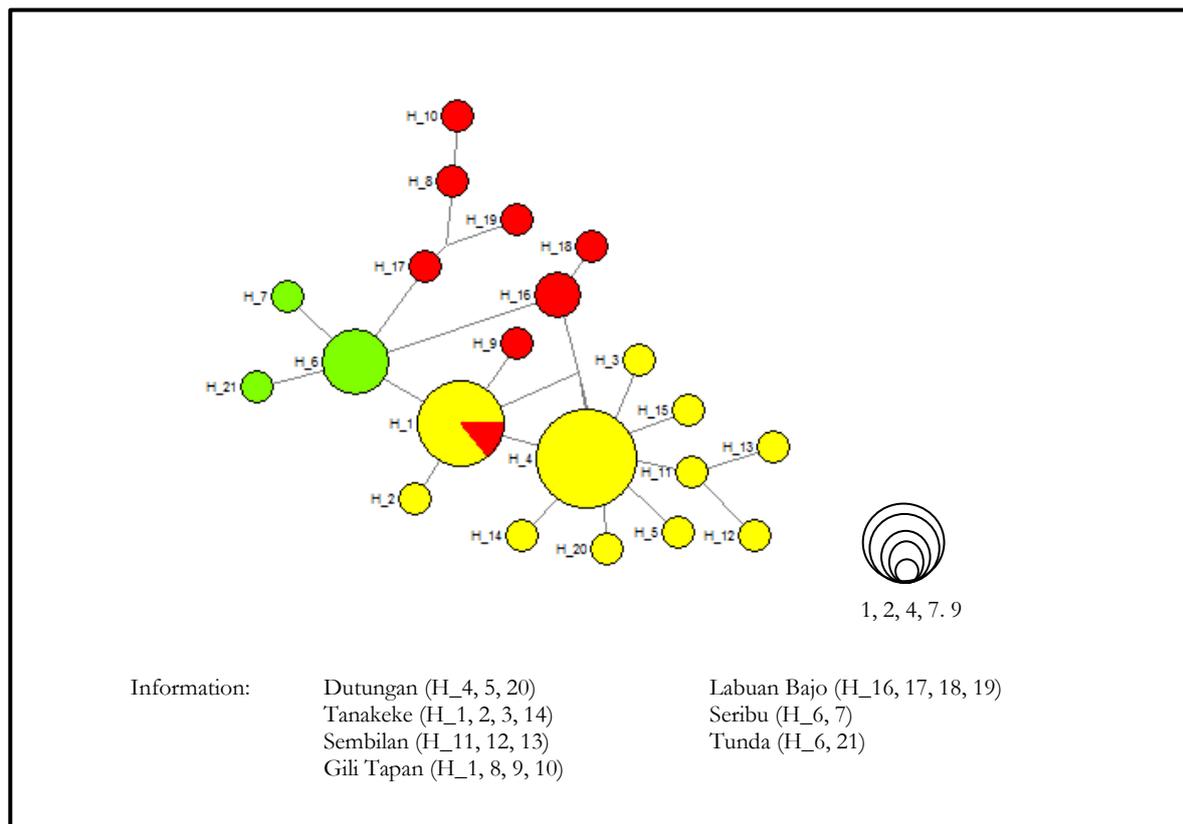


Figure 2. Haplotype network within the population on Java (Green), Sulawesi (Yellow), and Nusa Tenggara (Red).

Table 1. Genetic distance within populations and between populations in Sulawesi, Java, and Nusa Tenggara

Genetic distance	Lokasi	Java	Sulawesi	Nusa Tenggara
Within populations	Java	0.002	-	-
	Sulawesi	-	0.019	-
	Nusa Tenggara	-	-	0.026
Between populations	Java	-	-	-
	Sulawesi	0.044	-	-
	Nusa Tenggara	0.013	0.039	-

The results showed that the population on Sulawesi and population on Nusa Tenggara have 0.39 genetic distances (Table 1), and 0.399 Fixation index (Table 2). The values of genetic distance and genetic flow between both these populations was categorized as moderate compared with other populations (Sulawesi-Java and Nusa Tenggara-Java). Genetic distances between the population of Sulawesi and Java showed 0.044 and 0.558 for genetic flow on these populations. The values of genetic distance and genetic flow in both populations are the highest compared with other populations. While the smallest values of genetic distances and genetic flow between populations are Java and Nusa Tenggara's populations (0.013 and 0.227, respectively).

All populations have a close relationship of genetic distance and genetic flow. The range value of genetic distance 0010 - 0099 was categorized as low (Nei, 1972). The lower of genetic distance value indicated a close genetic relationship. The low value of genetic distance and high values of genetic flow on three

populations might be influenced by Indonesian Trough flow (Sulawesi - Nusa Tenggara) and seasonal currents (Nusa Tenggara - Java).

Table 2 *Fixation index (F_{ST})* in of Sulawesi, Jawa, and Nusa Tenggara.

F _{ST} Test	Location	Sulawesi	Java	Nusa Tenggara
	Sulawesi	-	-	-
	Jawa	0.558	-	-
	Nusa Tenggara	0.399	0.227	-

The result also found connectivity on each island in three populations. Dutungan Island is situated in the western of Sulawesi Island, the island is connected with Tanakeke Island which is thought be the center of *S. trocheliophorum*'s connectivity. The *S. trocheliophorum* have been connected from West to East Indonesia. The connectivity in the Eastern region (South Sulawesi) is connected between Tanakeke Island, Sembilan Island, and Gili Tapan. On the other hand, Labuan Bajo is also connected with Gili Tapan. The connectivity has also occurred in the western of Indonesia (Panggang and Tunda Island) which is also connected to Tanakeke and Gili Tapan (Figure 1 and 2).

Indonesian Through Flow have been flowing from the Pacific Ocean to the Indian Ocean (Gordon and Fine 1996) and is suspected of carrying the larvae of *S. trocheliophorum* from Dutungan Island to Tanakeke Island and Gili Tapan. The seasonal currents on the Java Sea and the Flores Sea created the connectivity between Tanakeke Island and Panggang Island, also allows the connectivity between Gili Tapan and Labuan Bajo.

The connectivity in three populations is expected because of nature gonochoric reproduction of *S. trocheliophorum* (Hellstrom *et al.*, 2010). The fertilization of sexual reproduction was external, this way could distribute the larvae. Larvae of *Sarcophyton* sp. are static and able to survive for 14 days on the water to spread following the flow of the waters (Benayahu and Loya 1986). It would be great opportunities to larvae of *S. trocheliophorum* to be distributed and settled far away from origin population. The current flow is suspected of spreading the larvae of *S. trocheliophorum* to various populations and develop of mixing between populations, such as between populations of Tanakeke - Gili Tapan. Mixing occurs in the population of Tanakeke possibly not only by Arlindo currents where coming from the Pacific Ocean, but also by seasonal currents flows from the Flores Sea.

Connectivity does not occur on the population of *S. trocheliophorum* at Panggang Island and Dutungan Island. This is presumably because the Arlindo current is more involved in the process of larval dispersal rather than in seasonal currents from the Makassar Strait. Moreover, the distance between those Islands is far enough, so that the larvae could not survive. The circulation flow pattern will provide information about the distribution of soft coral larvae, which therefore determine the pattern of connectivity.

Sembilan Island is located in the south of Sulawesi is the home of *S. trocheliophorum* which had a relationship with the population of Tanakeke Island, but they do not have a connectivity to Labuan Bajo and Gili Tapan, although those located are not too far away. This is possible because of the demographics of Sembilan Island where entered into the Bone Bay. The demographics of Sembilan island might be caused seasonal currents from Flores sea could not distribute the larva from Sembilan Island to Labuan Bajo and Gili Tapan.

Implication on Marine Protected Area

The population of Sulawesi especially Tanakeke island is the center of various Island in western and eastern Indonesia (Figure 2). Therefore, Tanakeke island needs more attention as the center of genetic connectivity. Tanakeke island could be supplying larvae to the western and eastern region in Indonesia. Changes in spatial and temporal scales can be used as coral reef ecosystem management implications, especially for designing marine protected areas (Munday *et al.*, 2009).

The damage to coral reef ecosystem in small islands in Indonesia could be impacted onto declining of marine biodiversity. It might be caused by anthropogenic activities, marine pollution, destructive fishing gear, and stolen of fish. While, The weakness of controlling and management of small islands in Indonesia also have the negative effect for declining of marine biodiversity. Eco-friendly management and utilization of *S. trocheliophorum* could be applied to save their population in the environment. The protection of genetic connectivity between population on each island in Indonesia can help the distribute of soft coral. Moreover, the designing of adaptive marine protected areas that connected by genetic to establish strong carrying capacity of ecology as spawning and nursery ground.

Establishment of marine protected areas in Indonesia was regulated on the regulation number 32 of 1990. At present, the establishment of marine protected area in Indonesia is only based on regional, while the establishment of marine protected area that involve on genetic connectivity between regions is still lacking. The genetic population which is well maintained can increase the genetic diversity. This would happen because of the smoothness of genetic transfer between the two populations were crossed.

CONCLUSIONS

The genetic structures of the population in all the population have low values, indicating the close genetic connection. Genetic connectivity connected on Population of Java, Sulawesi, and Nusa Tenggara. The connectivity between three populations is expected to become a reference in the process of forming marine protected area in Indonesia. Tanakeke as the center of genetic connectivity should get more attention to still be supplying the larvae of *S. trocheliophorum* to other population.

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