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Unexpected patterns of genetic connectivity in *Lobophyllia corymbosa* (Forskål, 1775) around Sulawesi, Indonesia

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5

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Abstract. Umar W, Tassakka ACMAR, Barber PH, Jompa J. 2019. Unexpected patterns of genetic connectivity in *Lobophyllia corymbosa* (Forskål, 1775) around Sulawesi, Indonesia. *Biodiversitas* 20: 2744-2749. Sulawesi is a strategic area for the biological networks of marine organisms, especially for Scleractinian corals. Sulawesi is located centrally in Indonesia and influenced by Pacific and Indian Ocean currents and shown in unique biodiversity patterns. However, research in this region is very limited. The wide distribution and lack of information on dispersal and connectivity make *Lobophyllia corymbosa* an exciting candidate in this study for genetic connectivity at the heart of the Indonesian Coral Triangle Area (ICTA), using Mitochondrial DNA (mtDNA) genome. Sampling sites (Palu, Mamuju, Sinjai, and Luwuk Banggai) with different coral fragments were collected from each site, DNA extracted, and mitochondrial COI gene was amplified. The results of the analysis were unexpected, and we hypothesize that the observed pattern was due to the evolution of different lineages within this region. The distance index and genetic diversity showed a close relationship between Mamuju and Luwuk Banggai, while Sinjai and Palu had a different genetic pattern. The global current patterns, complicated geography, and habitat condition could lead to such an outcome. Hard coral larvae arriving in an area might bypass a number of possible "stepping stones", resulting in unique patterns and leading to unexpected genetic connections within and between areas.

Keywords: Connectivity patterns, genetic variation, mitochondrial, scleractinian coral, Sulawesi

INTRODUCTION

The Coral Triangle is the global epicentre of marine biodiversity and Indonesia is the heart of this region. Biodiversity in Indonesia reaches its highest at the very west part - Eastern Islands of West Papua, and its lowest at the east part - Western Islands of Java and Sumatra (Roberts et al. 2002; Bellwood and Meyer 2009). Between these two regions, lies a group of Islands of Sulawesi. Sulawesi formed through island integration over the last 5 million years and its western, eastern and shores experience very different oceanography. Western Sulawesi borders the Indonesian Through Flow (ITF), the primary pathway for water transport from the Pacific to the Indian Ocean where currents can exceed 1m/s, while the eastern shores border the Maluku and Banda Seas, where there is little north to south transport (Gordon and Fine 2006). Modelling of oceanographic currents in this region (Tremblay et al. 2012; Kool et al. 2013) suggests that these different current regimes should have a significant impact on larval dispersal.

Many studies have used genetic data to infer patterns of larval dispersal within Indonesia (Barber et al. 2000, 2002, 2006; Timm et al. 2017; DeBoer and Barber 2010; Crandall et al. 2010). These studies typically identify three unique regions: (i) Western Indonesia, including Sumatera, and Java, (ii) Central Indonesia including the Lesser of Sunda

Islands, Sulawesi, and Sangihe-Talaud, and (iii) Eastern Indonesia, including Halmahera and West Papua (Barber et al. 2011; Carpenter et al. 2011). However, rarely these studies sampled on both sides of Sulawesi, and when they do, sampling has focused on the isolated islands in the Bay of Tomini.

The condition of coral reefs is declining worldwide, including Indonesia, and up to 85% of the damage occurring at the site is due to the anthropogenic activity (Burke et al. 2002). This degradation is concerning because of the economic and biological value of coral reefs (Cesar et al. 2003). Previous population genetic studies of corals in Indonesia have shown high levels of genetic differentiation, often on small spatial scales (Starger et al. 2010), suggesting that both genetic and demographic connectivity are limited (Hedgecock et al. 2007). As such, coral populations, the foundation of reef ecosystems, could be slow to recover from local disturbance. This fact is of particular interest in Sulawesi as the unique oceanographic regimes on its west and east coasts that could result in very different connectivity patterns around the island. However, to date, no coral studies have sampled on both sides of Sulawesi.

Lobophyllia corymbosa is a common coral around Sulawesi that traded as an ornamental coral. Although the International Union for Conservation of Nature (IUCN) lists this coral as a species Least Concern (Turak et al.

2014), *L. corymbosa* is a broadcast spawner coral (Coexploration 2017), making it a good model for understanding variation in genetic connectivity around the island of Sulawesi. Besides, it is only has reported from Sulawesi, the Seribu Archipelago and West Nusa Tenggara, making Sulawesi the northernmost portion of the species range so that patterns around Sulawesi cannot be influenced by upstream larval transport. In this study, we expand the geographic sampling to cover the western and eastern shored and use mtDNA sequences, which have been particularly useful in coral studies (Vollmer and Palumbi 2007) to determine patterns of genetic differentiation and connectivity.

MATERIALS AND METHODS

Sample collection

Field sampling was conducted during February-March 2017 and March at 2018, and then in four locations around Sulawesi, we collected 20 fragments from Palu, 13 from Mamuju, 15 samples from Sinjai and also 15 from Luwuk Banggai. The sites were determined based on the geographical form of Sulawesi and its surrounding waters, as well as the lack of data on genetic connectivity in those areas (Figure 1). Samples (*L. corymbosa* fragments) from different colonies were collected at each site at an average depth of 3-7 meters using a hammer and chisel. Within each population (location), sampling was random, with a distance of at least 2-10 meters between colonies to

minimise the likelihood of collecting clones (Van der Ven et al. 2016; Hemond and Vollmer 2010). After collection, the samples were preserved in 95%-100% laboratory grade ethanol in individually labelled containers and stored at room temperature for further analysis (Huang et al. 2011; Timm et al. 2017).

Procedures

Primer design and selection-1

Primer design and selection referred to accession number AB117241.1 in the NCBI Web (National Centre for Biotechnology Information). Specific mitochondrial Cytochrome Oxidase I (COI) primers were designed and tested using BLAST to ensure their suitability for the target samples. Potential primer selection aimed to find a suitable pair which could be amplified from the samples. The primer pair selected was: mtCOI CAGG CGCT ATGT TAGG AGATG as a forward and mtCOI CCGG CTAATACA GGCA AAG ATA as a reverse. In this research, we used the mitochondrial genome as a target, because of ancestor information can be defined from that marker, from this, it will provide connectivity information that flows to an organism.

DNA extraction, PCR, electrophoresis, and sequencing-2

Genomic coral DNA was extracted using a commercial extraction kit (Qiagen Blood and Tissue Kit); procedures followed the protocols used in previous research (Huang et al. 2011). Coral tissue was retrieved by crushing the coral fragments to obtain a 0.10 g sub-sample from each sample. Following the protocol, the samples were incubated in the Qiagen kit lysis solution in a heat block at 56°C for 24 hours (Van der Ven et al. 2016). The proprietary extraction processes (protocols) were then followed to obtain DNA genomic extracts, which were then stored in a freezer at -4°C (Cho and Shank 2010).

PCR was performed in 0.2 mL reaction tubes, using the M15 q RedMix (Bioline) reagent mix. Each tube contain a volume of 26 µL consisting of 10 µL ddH₂O, 1.25 µL each of the forward and reverse primers, 12.75 µL MyTaq RedMix (Bioline), and two µL of template DNA (Fukami et al. 2004). The thermocycler profile consisted of 38 cycle preceded by a pre-denaturation at 95°C for 15 min, with denaturation at 94 °C for 1 min, annealing at 53°C for 1 min, and extending at 72°C for 105 s, followed by a final step at 72°C for 10 min.

The presence or absence of a DNA band was determined through electrophoresis on an agarose bed (1% agarose gel, 0.75 g; 75 mL SB Buffer; 4 µL Ethidium Bromide) at 100 V/400 amp for 30 min. PCR products testing positive for DNA were then sent to the Sequencing Centre, Berkeley for Sanger sequencing (Kortschak et al. 2003). Sequences obtained were edited (to remove ambiguous bases at each end) and analysed in Molecular Evolutionary Genetic Analysis (MEGA) ver. 6 (Tamura et al. 2013), using the statistical method of Maximum Likelihood with 1000 bootstrap replicates (Tamura and Nei 1993) to infer the evolutionary relationships between the samples. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) in units of

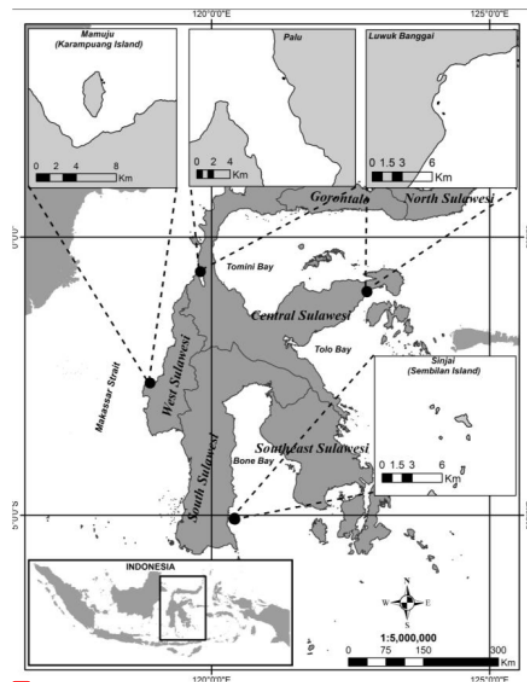


Figure 1. Map of research sites in Sulawesi waters, Indonesia

29
a number of base substitutions per site. All positions were 25 ed and eliminated for further analysis. Evolutionary analyses were conducted in MEGA 6 (Tamura et al. 2013) and haplotype diversity analysis was implemented in DnaSp ver. 5.10 (Librado and Rozas 2009).

RESULTS AND DISCUSSION

Results

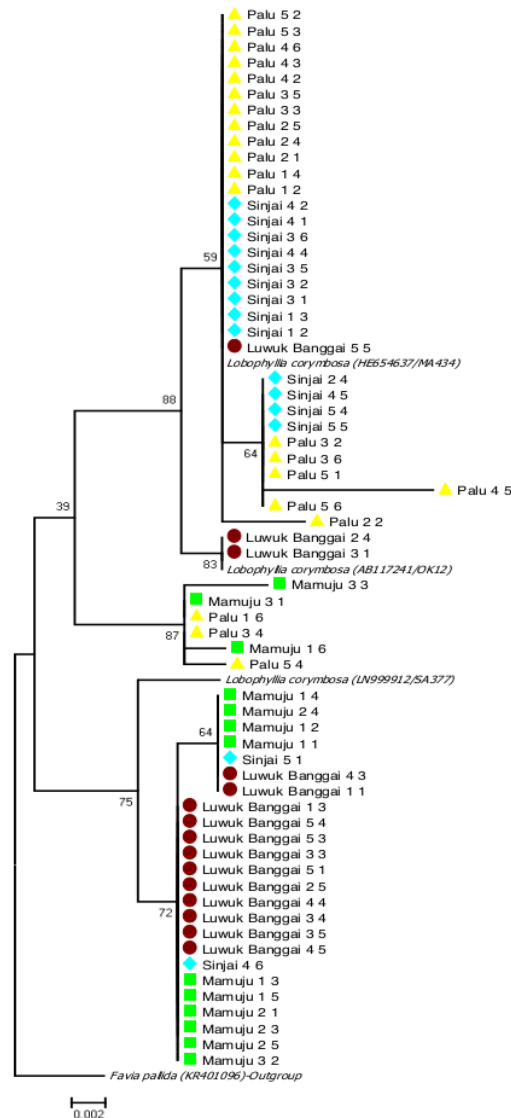
The mitochondrial genomic material derived from each of the 64 nucleotide samples was successfully amplified and produced an informative sequencing product. The nucleotide base was trimmed with 437 base pairs (bp) in length. The sequences of all samples had a similarity of 99%-100% with the *L. corymbosa* sequence on Genebank with 2 accession number AB117241 (Fukami et al. 2004).

The optimal phylogenetic tree with the sum of branch length = 0.002 (Figure 2) showed a pattern of two significant clusters formed from the four *L. corymbosa* populations (Palu-Sinjai and Mamuju-Luwuk Banggai). Genetic distance values obtained from distance method in pairwise difference. The values indicated that for connectivity, locally, they had a very close relationship. However, when comparing them with index D on intra-population, the unexpected happened to the population of Mamuju and Luwuk Banggai. Their genetic distance was 0.037, as well as the value formed in Sinjai and Palu population ranges from 0.010, which was not much different from the previous populations. While the genetic distance formed in geographically possible areas to have a close relationship with Palu, Mamuju, and Sinjai showed a D value that tended to be high (Table 1).

In addition to genetic distance analysis, the results of the haplotype diversity analysis showed the distribution of diversity and haplotypes within each population (Table 2). A total number of 11 *L. corymbosa* haplotypes were identified, with a geographical distribution shown in Figure 3. Mamuju and Luwuk Banggai areas shared haplotypes (1 and 2), as did Palu and Sinjai that shared one significant haplotype in yellow one (haplotype 4). Mamuju and Palu, the two sites closest to each other, only shared one haplotype (7). Besides, one haplotype was only found in Luwuk Banggai (3), while Palu had three unique haplotypes (9, 10, and 11) and neither in Mamuju with two specific haplotypes (6 and 8).

1
Table 1. The number of base substitutions per site from averaging over all sequence pairs between populations are shown in distance method of pairwise difference

| Site | Luwuk Banggai | Sinjai | Mamuju | Palu |
|---------------|---------------|--------|--------|------|
| Luwuk Banggai | | | | |
| Sinjai | 0.566 | | | |
| Mamuju | 0.037 | 0.577 | | |
| Palu | 0.604 | 0.010 | 0.603 | |



7
Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the 14 Maximum Likelihood method based on the Tamura-Nei model (1993). The tree with the highest log likelihood (-21.0636) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. 3 trial tree for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, 14 parameter = 0.1000)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 12.7630% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6.

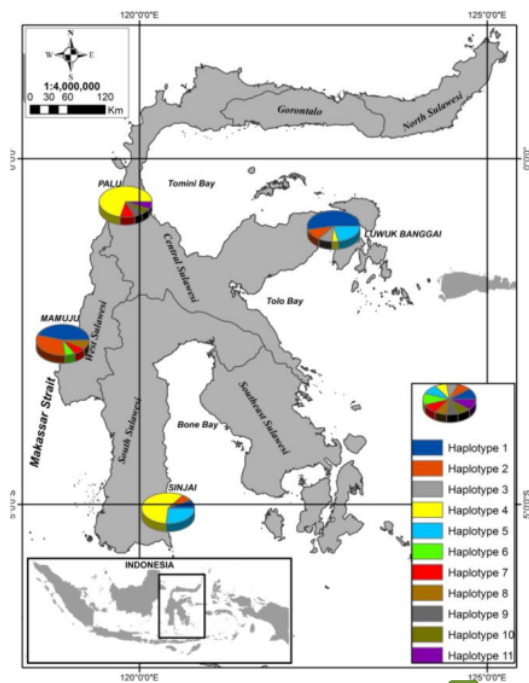


Figure 3. Haplotype map for *Lobophyllia corymbosa*. Pie graphs indicate haplotypic composition of each location/population

Table 2. Haplotype information each site defined

| Site | Haplotype (h) | Haplotype diversity (Hd) | Nucleotide diversity |
|----------------|---------------|--------------------------|----------------------|
| Luwuk, Banggai | 4 | 0.554 | 0.006 |
| Sinjai | 4 | 0.600 | 0.005 |
| Mamuju | 5 | 0.730 | 0.008 |
| Palu | 6 | 0.652 | 0.005 |

Table 3. Genetic structure analyses were conducted using AMOVA in Arlequin

| Source of variation | d.f | Sum of squares | Variance components | Percentage of variance |
|---------------------|-------|----------------|---------------------|------------------------|
| Among population | 3 | 66.270 | 1.31150 Va | 49.24 |
| Within population | 60 | 81.105 | 1.35176 Vb | 50.76 |
| Total | 63 | | 2.63326 | |
| Fixation index | 0.492 | | | |

The distribution of haplotypes within each population was similar. Luwuk Banggai and Sinjai had four haplotypes for each site, while Mamuju has five haplotypes and the higher one from Palu with six haplotypes. The

haplotype diversity (Hd) depended on the number of haplotypes were found, in populations with a relatively high number of haplotypes (h), i.e. Palu and Mamuju, the Hd values were 0.652 and 0.730 respectively, while Luwuk Banggai and Sinjai had lowest Hd values (0.553 and 0.600, respectively). To investigate population structure, pairwise Fst-values were estimated through an Analyses Molecular Variance ver. 3. 5.2.2 (AMOVA) (Excoffier et al. 2005), yielding a value of 0.492 (Table 3), indicating a moderate level of connection.

Discussion

Generally, in a region, the population has a close kinship value if it is in the same line, but this study found unusual connectivity patterns. Investigation of genetic connectivity information in the same species (*L. corymbosa*) for four sites on a geographical basis visible enough to have a close relationship, but the results obtained did not show a harmonised congruent. The previous study by Cho and Shank (2010) presented the result which had connectivity pattern. It was not connected to Ophiroid species in Northern Atlantic even though the place geographically was not in an isolated area. Also, studied by Timm et al. (2017) in Spermonde Archipelago of Indonesia described the genetic information of coral population structures. It found that genetic flows were limited to their research sites although geographically still on a small scale, it was assumed that high recruitment in the area and weak currents also become a limitation in the distribution of gene material. While on a large extent, they found the closeness was close enough, this was also what happened in this study.

Location of Sulawesi, the central of Indonesia received a robust current flow from the Pacific Ocean to the Indian Ocean, are the vector flow towards the West and East waters of Sulawesi (Gordon and Fine 1996), allowing for a close genetic distance which occurred in both populations (Mamuju and Luwuk Banggai). Moreover, the *L. corymbosa* as a coral with a broadcast spawning reproduction type (Richmond and Hunter 1990) meaning that they had a more extended time period on the water column until settlement time to a suitable substrate making the species expected to perform "stepping stone" on islands or areas far from "home" to allow for the spread of coral larvae far enough. Meanwhile, the distance along the coastline from Palu to Mamuju ranged from 248 km, not strong enough connection seen between these two populations, although both were quite close. The role of the current mentioned earlier was quite influential, especially the current of the Makassar Strait which directly traversed to the two populations, thus allowing the genes flew that form and became a barrier to the population. On the contrary, the genetic flow formed between Sinjai and Palu looked quite close even though it was about 856 km away. This also occurred in connectivity research on *Acropora cervicornis* in Florida. The results showed that although the Florida and Bahamas area had proximity (200 km) reasonably, no significant genetic relationship existed between the two populations (Hemond and Vollmer 2010). It was also mentioned in Wood et al. (2014), to simulate

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