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Genetic diversity and population structure of the boring giant clam (*Tridacna crocea*) in Kei Islands, Maluku, Indonesia

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Abstract. *Kusnadi A, Kurnianto D, Madduppa H, Zamani NP, Ibrahim PS, Hernawan UE, Utami RT, Triandiza T. 2022. Genetic diversity and population structure of the boring giant clam (*Tridacna crocea*) in Kei Islands, Maluku, Indonesia. Biodiversitas 23: 1273-1282.* Giant clams (Tridacninae) are ecologically important species in the coral reef ecosystems. They provide valuable functions to traditional fisheries in Kei Islands, Maluku. However, their population is under great pressure due to anthropogenic threats, such as overfishing and habitat degradation. To provide important data for devising effective conservation management strategies for giant clams, we investigated genetic diversity and population structure of the boring giant clams *Tridacna crocea* in Kei Islands based on partial mitochondrial COI gene sequence. Tissue samples were collected from six sites: Kur, Dullah Laut, Tanimbar Kei, Dar, Labetawi, and Difur. We sequenced 477 base pairs of COI gene and identified 42 haplotypes and 52 polymorphic sites. Analysis of genetic diversity showed Dullah Laut and Dar had the highest genetic diversity. Population structure and genetic distance analysis showed unstructured populations with high genetic closeness among sites. This finding was also confirmed by the mixture pattern of the haplotype network. Further analysis using Bayesian models on gene flow revealed high genetic exchange among sites and that Dullah Population predominantly served as a source site for the other sites. This indicated a high probability of successful larval dispersal among sites. Based on these findings, we predict that the boring giant clams likely form a single population in Kei Islands. Our study warrants conservation priority for Dullah population as the main source of gene flow.

Keywords: Bayesian model, COI gene, conservation priority, gene flow, Kei Islands

INTRODUCTION

Giant clams (Tridacninae) are the largest bivalves associated with coral reef ecosystems in the Indo Pacific (Lucas 2014; Neo et al. 2017), which are ecologically important due to their contribution to ecosystems functions (Soo and Todd 2014; Vicentuan-Cabaitan et al. 2014; Neo et al. 2015). These species are ecosystem engineers and a natural bio-filter that controls eutrophication by filtering dissolved ammonia and nitrate (Klumpp and Griffiths 1994; Neo et al. 2015). Moreover, giant clams are economically important species that are intensively harvested for food and marine aquarium trade markets in Japan, Hongkong, Australia, and the USA (Wabnitz et al. 2003; Nijman et al. 2015). Similarly, their shells are used as raw materials in the ceramic handicraft industry (Neo and Loh 2014; Nijman et al. 2015; Larson 2016; Mies et al. 2017; Lyons et al. 2018).

Meanwhile, overfishing has led to a drastic decline in the population of giant clams in the Indo-Pacific, specifically in Indonesia (Larson 2016; Neo et al. 2017). Recent studies showed that low population density with less than 0.1 individual/m^2 in several parts of Indonesia (Naguit et al. 2012; Hasni et al. 2017; Ode 2017; Wakum et al. 2017; Harahap et al. 2018; Triandiza et al. 2019; Rizkifar et al. 2019).

Overfishing of giant clam populations also leads to a decrease in genetic diversity, which affects population sustainability and species persistence under changing environmental conditions (Kahilainen et al. 2014; Madduppa et al. 2014. Meanwhile, genetic diversity and gene flow among populations influence species' capacity in adapting to environmental change (Jena et al. 2011; Bonde et al. 2012). Therefore, understanding the pattern of genetic diversity and gene flow is of great concern to conservation management. By identifying this pattern among geographically separated populations, managers facilitate conservation strategies, such as designing spatial units to conserve, identification of the source, and sink populations, and site determination (Dauphinais et al. 2018)

Previous study about Genetic population structure was held in the coral triangle (DeBoer et al. 2014). Data regarding the genetic structure of the giant clam population in Kei Islands is still not available. Previous studies have shown that the occurrence of giant clams in the Kei Islands in the southeastern part of Maluku, Indonesia (Hernawan 2010; Triandiza et al. 2019) provide livelihood benefits to the local people. Furthermore, fishermen harvest giant

clams for local consumption, export, and construction material (Kusnadi et al. 2008). A previous study by Hernawan (2010) stated that the giant clam population had very low density, of approximately 50 individuals/ha. This is due to the anthropogenic pressures and emphasized effective conservation strategies for these unique, but threatened bivalves in Kei Islands. Furthermore, the site selection in the Kei Islands is based on the existence of a conservation area in the area. Tanimbar Kei is a conservation area in accordance with the Decree of the Minister of Marine Affairs and Fisheries Number 6/KEPMEN-KP/2016 and Dullah Laut as a conservation area in accordance with the Decree of the Mayor of Tual Number 407 of 2015. Therefore, this study aims to determine the genetic diversity and population structure of the boring giant clams (*Tridacna crocea)* in Kei Islands to provide important data for devising effective conservation management strategies.

MATERIALS AND METHODS

Study sites

This study was conducted from October 2017 to September 2018, while the fieldwork for collecting giant clam tissue was carried out at 6 sites, namely Dar station (-5.72941666666666°S; 132.80222222222224°E), Kur Island (-5.305691666666666°S; 132.01265555555556°E), Tanimbar Kei (-5.987025°S; 132.4648°E), Dullah Laut Island (-5.551638888888888°S; 132.74533333333332°E), Difur (-5.5488°S; 132.7985361111111°E), and Labetawi village (-5.554361111111111°S; 132.77825°E), Maluku, Indonesia (Figure 1). Furthermore, the study sites were determined based on the reports from local giant clam fishermen and previous research (Hernawan 2010; Triandiza et al. 2019). Sampling was implemented once for each site. Molecular analysis was conducted at the Marine Biodiversity and Biosystematics Laboratory, Faculty of Marine and Fisheries, Bogor Agricultural University.

Tissue sample collection and handling

Samples were collected by using non-destructive methods after the permission was granted from the local leader. The number of samples varied for each location ranged from 8 samples to 24 samples. The samples were identified to species level based on morphological characteristics using the references (Copland and Lucas 1988; Norton and Jones 1992). Pictures of giant clams were taken on site and labeled to verify identification or for future reference. Specimens varied in size from juveniles to adults ranging from 1.0 cm to 16 cm. Mantle tissue about 1 cm length was collected from each sample and placed in 1.5 mL microcentrifuge tubes in 95% ethanol for preservation.

Isolation and extraction of total DNA

DNA from the preserved mantle tissue samples were extracted using the commercial kit GeneAid. A total of 25 mg of tissues was cut into small pieces and processed with the kit according to the manufacturer's instructions. Since all samples were morphologically identified as either *T. crocea*. Sequences of the MT-CO1 gene were PCR-amplified using the MT-CO1 universal (forward: LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse: HCO2198: 5'-TAAACTTCAGGGTGACC AAAAAATCA-3) (Folmer et al. 1994) and tridacnid-specific primers (forward: LCO: 5'-GGGTGATAATTCG-AACAGAA-3' and reverse: RCO: 5'-TAGTTAAAGCCCCAGCTAAA-3') (Nuryanto et al. 2007). Total volume of reaction was 27 µL which consisted of 3 µL of DNA template, 12.5 µL of Thai TAQ buffer, 1.25 µL of each primer and 9 µL ddH2O.

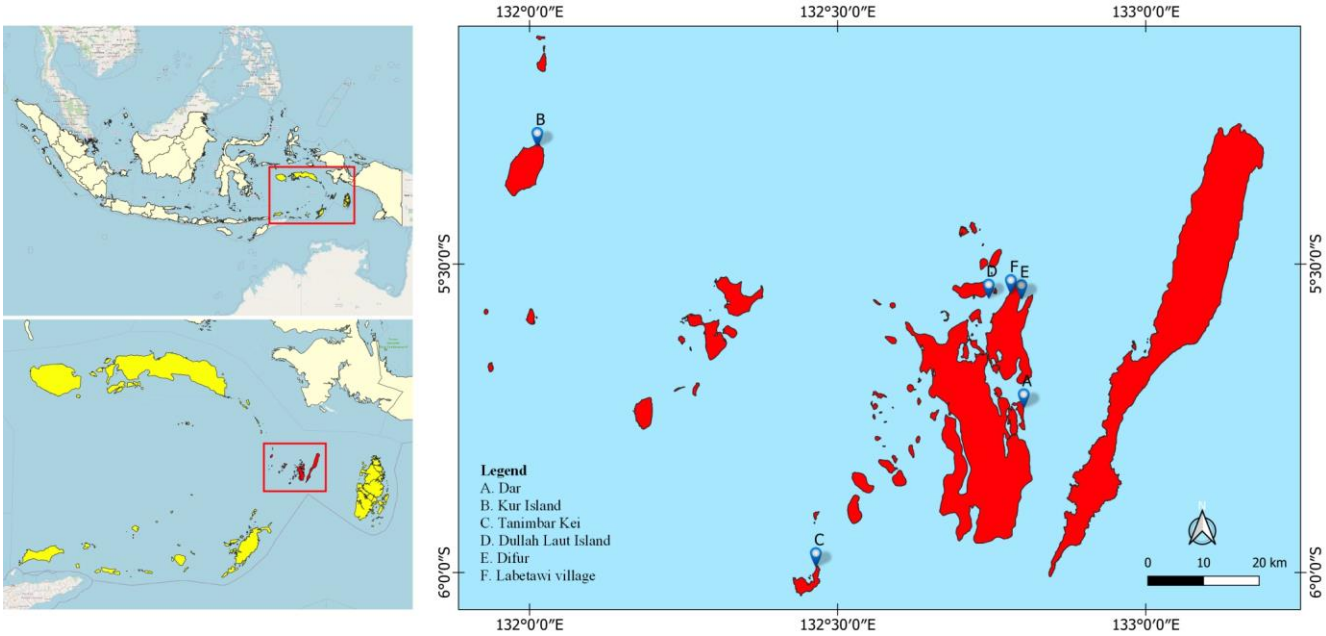

Figure 1. Sampling localities of *Tridacna crocea* across the Kei Islands, Maluku, Indonesia

PCR amplification

Amplification was done by using PCR with predenaturating condition at 94°C for 5 minutes, continued with 35 cycle consist of 1 minute of denaturation at 94°C, annealing at 43°C for 90 second and first extension at 72°C for 1 minute and 5 minutes last extension at 72°C.

DNA sequencing

The result of PCR amplification is then tested for its quality through electrophoresis. This technique used agarose gel with concentration 1.0% diluted on Tris Acetate EDTA and Ethidium Bromide (EtBr). The PCR results were prepared in agarose wells, then electrophoresis was performed using a voltage of 100 volts for 25 minutes. The low mass DNA ladder (invitrogen) was used to determine the DNA strand length which was viewable in *Geldoc* using a UV transilluminator. A good PCR result will show a clear band with a product size of 500-700bp (base pairs). Successfully amplified products were sent to First Base Malaysia by the Sanger method for sequencing (Sanger et al. 1977).

Data analysis

The sequencing data was stratified with *Clustal W* program on MEGA 6 (*Moleculer Evolutionary Genetic Analysis*) software (Tamura et al. 2013). DNA sequence from this study is compared with DNA sequence from GenBank to ensure that those species are *Tridacna crocea*. Neutral mutation hypothesis testing on DNA polymorphisms was carried out using the Tajima test (1989) and the Fu and Li test (1993). Haplotype diversity (Hd) (Nei 1987), Nucleotide diversity (π) (Nei and Jin 1989), and population structure was analyzed with Arlequin (Excoffier and Lischer 2010). A median joining network of mtCOI haplotypes based on pairwise nucleotide differences was also constructed with Network 4.6.1.0 using default settings (Bandelt et al. 1999). To infer genetic differentiation among populations, an analysis of molecular variance (AMOVA) was estimated by Fixation index (*Fst*) based on pairwise genetic distances using Arlequin 3.5.2.2 software (Excoffier and Lischer 2010). The relationship of haplotype phylogenetic was analyzed by using network software 5.0.0.3 (http://www.fluxusengineering.com). Gen flow on *T. crocea* population analyzed by using Bayesian inference on Migrate-n program 3.6.11 version (Beerli et al. 2019).

RESULTS AND DISCUSSION

Genetic diversity

The DNA amplification result through PCR obtained a total of 493-684bp fragment length. It occurs because the amplification process uses 2 types of primers, namely universal CO1 primer and special CO1 primer for giant clams. while after aligning using the *Clustal W* program on MEGA 6, only 477 bp of DNA fragments were obtained and utilized for further analysis. Furthermore, homology analysis based on BLAST-N on *Cytochrome oxidase* I (COI) mtDNA of *T. crocea* showed 98-99% (expected values >97%) similarity to the GenBank sequences. The results of the genetic diversity analysis of 101 samples of *T. crocea* using the Dnasp program are shown in Table 1. Based on these results, Dullah and Dar have the most genetic diversity, indicating the most haplotype diversity (Hd) and nucleotide diversity (π) values (Hd= 0.9722 ± 0.0640 and 0.9708 ± 0.0273; π= 0.0118 ± 0.0066 and 0.01097 ± 0.0067), while the lowest genetic diversity was in Tanimbar Kei (Hd= 0.8116 ± 0.0584; π= 0.0078 ± 0.0045).

The haplotype distribution analysis results showed that the population of *T. crocea* in the Kei Islands had 30 unique and 12 mixed haplotypes between the populations (Figure 2). Meanwhile, the highest haplotype frequency was on H-4 (42.56%) with a distribution that covered almost all populations, except Dullah Laut. This was followed by H-12 (28.57%) with haplotype distribution which includes Dullah Laut, Dar, Tanimbar Kei, and Labetawi, moreover, all the populations have a unique haplotype. Dullah Laut is a population of *T. crocea* with the highest unique haplotype (10 haplotypes), while Dar has the lowest (1 haplotype).

Genetic population structure

All the paired F_{ST} values of the *T. crocea* population in Kei Islands were relatively low (below 0.07). Out of these paired populations, Tanimbar Kei showed relatively higher paired F_{ST} values, while Dar gave the lowest (Table 2). The paired F_{ST} analysis results showed that most of the population had no significant difference. The results of the population structure analysis of *T. crocea* using the AMOVA test (Excoffier et al. 1992) showed that there was no genetic structure between populations in the Kei Islands as indicated by a low paired F_{ST} value (F_{ST}= 0.0231; P-value= 0.0215) (Table 3). A previous study by Excoffier and Lischer (2010) stated that the F_{ST} value= 0 indicated that there is no genetic difference between populations, while the F_{ST}= 1 value indicated that there are differences in genetic characters. Since the F_{ST} values in this study were closer to the 0 value, therefore, there was no difference in the structure of the observed population. This showed that all observed populations have a low genetic difference.

Table 1. Genetic diversity of *Tridacna crocea* in Kei Islands in terms of Number of Haplotype (*Hn*), Haplotype diversity (*Hd*), and Nucleotide diversity (π)

Population (sites)	N	Genetic diversity		
		H_n	H_d	Π
Kur	8	6	0.8929± 0.1113	0.0084±0.0056
Dullah Laut	19	15	0.9708±0.0273	0.0118±0.0066
Labetawi	21	14	0.9429±0.0328	0.0097±0.0055
Tanimbar Kei	24	9	0.8116±0.0584	0.0078±0.0045
Difur	20	9	0.8579±0.0537	0.0083± 0.0048
Dar	9	8	0.9722±0.0640	0.01097±0.0067
Total	101	42	0.9426±0.013	0.0093±0.00055

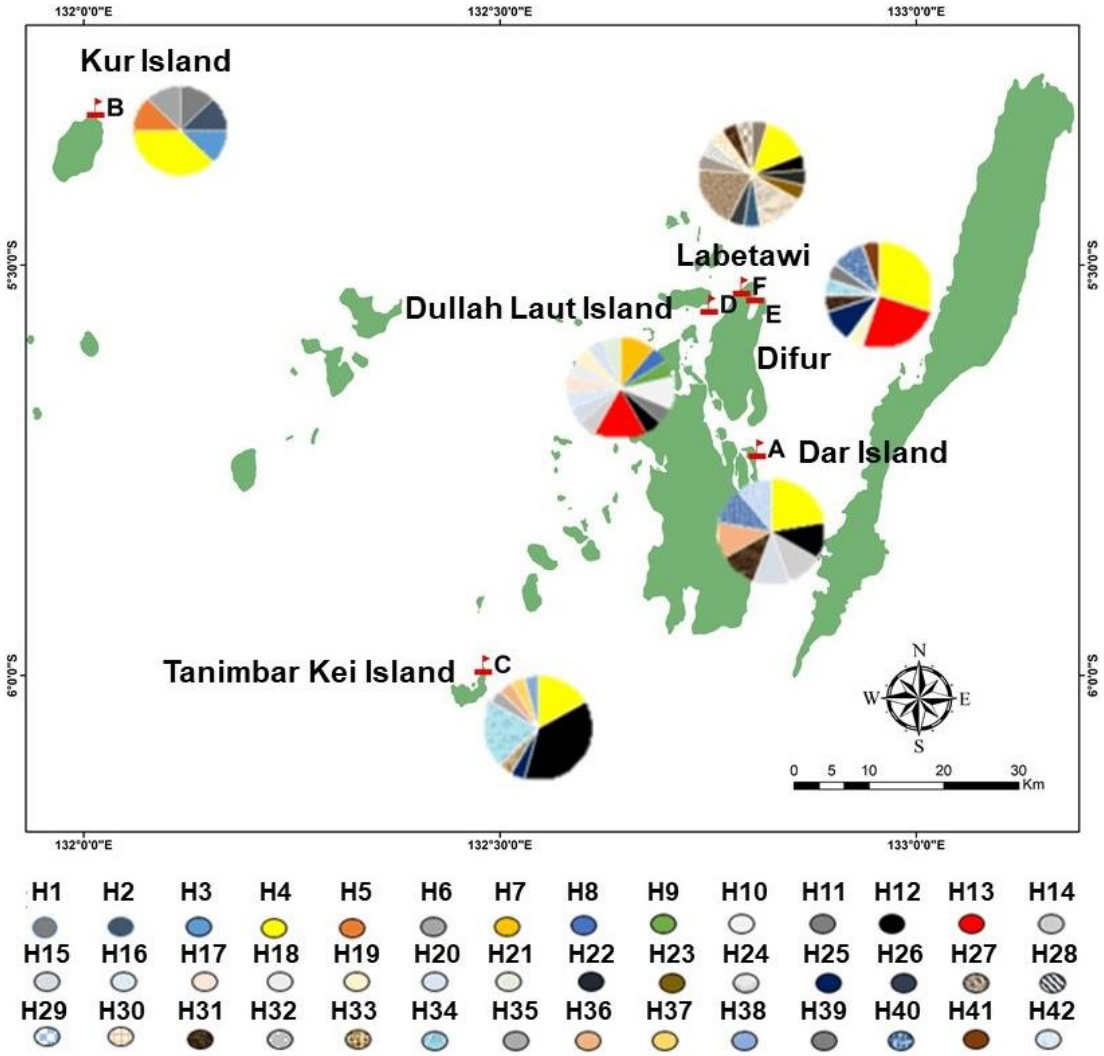

Figure 2. Distribusi haplotipe sekuen *Tridacna crocea* di Kei Islands, Maluku, Indonesia

Low genetic difference between populations of *T. crocea* indicated the genetic proximity. Meanwhile, the genetic proximity between *T. crocea* populations in the Kei Islands was represented by the low genetic distance (Table 4). Furthermore, genetic distance analysis shows the proximity within the intra and inter populations. The results of genetic distance analysis on intrapopulation of *T. crocea* ranged from 0.0077 (Tanimbar Kei) to 0.0110 (Dullah Laut) and the genetic distance was 0.0081-0.0107 and the closest genetic distance was between Difur and Kur with a value of 0.0081, while the farthest was the Labetawi and Dullah Laut populations (0.0107). A previous study by Nei (1972) stated that the genetic distance with a value of 0.010-0.099 was in a low category. Therefore, the lower value of the genetic distance, the closer the genetic relationship between these populations.

Genetic connectivity

The haplotype network analysis showed that there were three main groups (Figure 3), namely groups A, B, and C. Meanwhile, group A consisted of 4 haplotypes that are scattered throughout the *T. crocea* population in Kei Islands, where the dominant population was Difur with 2 haplotypes and 4 individuals. Group B consisted of 12 haplotypes in all *T. crocea* populations in Kei Islands. Haplotype 4 has the highest number of individuals (18 individuals) that are in 5 populations, except Dullah Laut, in group B which contains 9 unique haplotypes. Furthermore, haplotype 20 from Dullah Laut was the most distinctive that was limited by 5 mutations. Group C has the highest haplotypes which are 26 and spread out across all *T. crocea* populations in the Kei Islands. There are 4 dominant haplotypes in the population of Tanimbar Kei (H 12, 9 individuals), Labetawi (H27 and H24, 7 individuals), and Difur (H13, 5 individuals). Labetawi and Dullah Laut were the populations with the highest haplotypes in group C, namely 10 and 9. Also, haplotypes 17 and 23 were the most different in group C due to their limitations to 3 mutations. These results showed that there was haplotype mixing in all *T. crocea* populations in the Kei Islands as shown by no specific clade. The height of the unique haplotype in *T. crocea* populations in the Kei Islands suggests local genetic variation in the population.

Tabel 2. Inter population analysis of *Tridacna crocea* in Kei Islands, Maluku, Indonesia based on Pairwise *F*ST value (below diagonal) and P-value (above diagonal)

Location	Kur	Dullah Laut	Labetawi	Tanimbar Kei	Difur	Dar
Kur	-	0.2451^{ns}	0.2881^{ns}	0.083^{ns}	0.6455^{ns}	0.8027^{ns}
Dullah laut	0.0162	-	0.0527^{ns}	0.0147^{s}	0.0557^{ns}	0.749^{ns}
Labetawi	0.0172	0.034	-	0.0664^{ns}	0.0596^{ns}	0.7197^{ns}
Tanimbar Kei	0.05845	0.0642	0.0389	-	0.0176^{s}	0.3281^{ns}
Difur	-0.0329	0.0422	0.043	0.068	-	0.5303^{ns}
Dar	-0.04347	-0.0212	-0.0217	0.0089	-0.0182	-

Note: ns: not significant (P>0.05); s: significant (P<0.05)

Table 3. Analysis of molecular variation (Amova) of *Tridacna crocea* population in Kei Islands, Maluku, Indonesia

Source of variation	df	Sum of squares	Component of variance	Percentage of diversity	F_{ST}	P-value
Among population	3	8.102	-0.05019Va	-2.20	0.0231	0.0215±0.00462
Among individual within population	2	8.583	0.10360Vb	4.51		
Within individual	95	21.047	2.23208Vc	97.69		
Total	100	288.733	2.28495	100		

Table 4. Analysis of genetic distance Intra and Inter-population of *Tridacna crocea* in Kei Islands, Maluku, Indonesia

Genetic distance	Location	Kur	Dullah Laut	Labetawi	Difur	Dar	Tanimbar Kei
Intra-population	Kur	0.0085	-	-	-	-	-
	Dullah Laut		0.0110		-	-	-
	Labetawi	-	-	0.0099	-	-	
	Difur	-	-	-	0.0084		-
	Dar	-	-	-	-	0.0099	
	Tanimbar Kei	-	-	-		-	0.0077
Inter-population	Kur	-	-	-		-	-
	Dullah laut	0.0100	-	-	-	-	-
	Labetawi	0.0094	0.0107	-	-	-	-
	Difur	0.0081	0.0100	0.0095		-	-
	Dar	0.0088	0.0103	0.0096	0.0088		-
	Tanimbar Kei	0.0087	0.0098	0.0091	0.0086	0.0087	-

The occurrence of haplotype mixing shown by the formation of haplotype groups (Figure 3) indicated the existence of gene flow through genetic structure between *T. crocea* populations in the Kei Islands, namely Pulau Kur, Dullah Laut, Labetawi, Difur, Dar, and Tanimbar Kei. Moreover, the pattern of larval dispersal of *T. crocea* gene flow in the Kei Islands using Bayesian analysis (Beerli 2006; Beerli 2009) showed that the rate of migration was 6.12-58.76 with the highest gene flow from the Dullah population (M1), while the lowest was from Dar (M2) to other populations in the Kei Islands (Table 5). This showed that the Dullah Laut population (M1) acted as a source because it supplied more gene flow to other populations, while Dar served as a sink population because it received more gene flow from other populations (Figure 4).

Table 5. Gene flow at four *T. crocea* populations in Kei Islands, Maluku, Indonesia based on Bayesian analysis (M1: Population Dullah (Dullah Laut, Labetawi, and Difur), M2: Dar, M3: Kur Island M4: Tanimbar Kei Islands)

Location	M1	M2	M3	M4
M1	-	58.67	34.03	42.15
M2	6.12	-	12.09	10.36
M3	29.64	12.64	-	15.69
M4	27.24	28.89	16.8	-

Note: Gene flow rate from source population to sink population was indicated by values above the diagonal (bold), whereas gene flow rate from sink population to source population was indicated by values below the diagonal

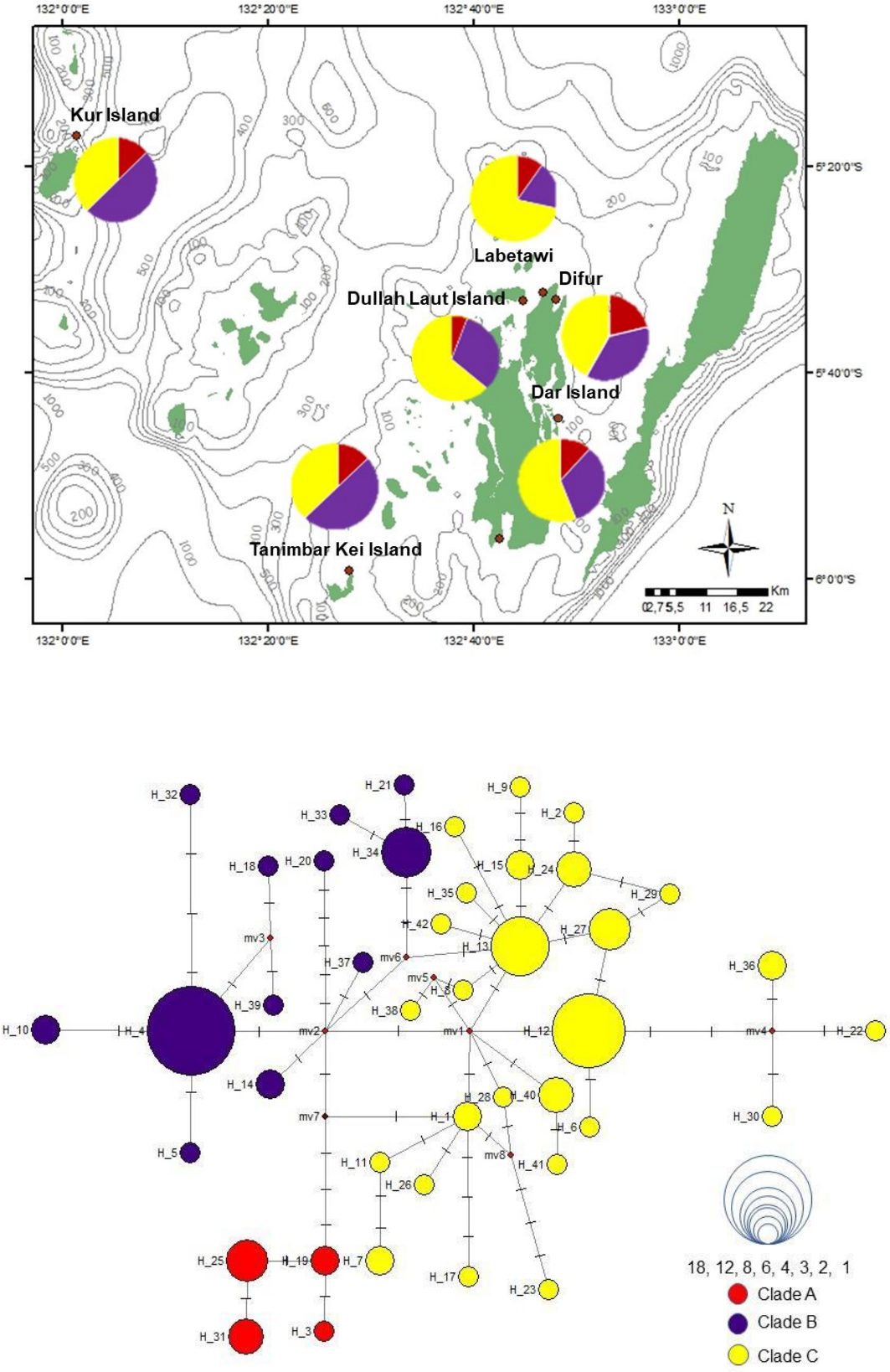

Figure 3. Connectivity of 42 haplotypes from 101 samples of *Tridacna crocea* in Kei Islands, Maluku, Indonesia by using the median-joining method. Note: location signed with color, every haplotype represented by a circle, circle size indicated the frequency of haplotype

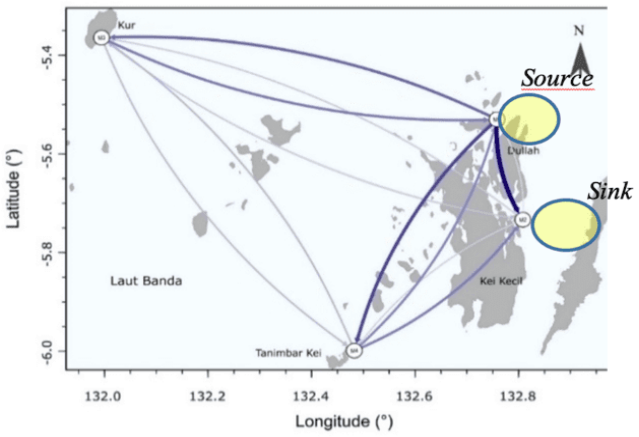

Figure 4. Visualization of gene flow at four *T. Crocea* population in Kei Islands, Maluku, Indonesia. The source population is the population that acts as a donor of gene flow to the environment whereas the sink population play a role as recipient of the gene flow from the source population

Discussion

Previous studies about Genetic population structure in the Indo-Malay archipelago (Kochzius and Nuryanto 2008; de Boor et al. 2008), Indo-West Pacific (Hui et al. 2016), and the coral triangle (DeBoer et al. 2014) showed a very strong genetic population structure and a complex connectivity pattern, characterized by limited gene flow in almost all sample locations. Furthermore, geographical distance isolation patterns, geological history, and oceanographic conditions all contribute to shaping the genetic population structure and limited gene flow in the *T. crocea* population (Hui et al. 2016). Crispo and Chapman (2008) stated that ecological isolation and geographic structure influence the genetic population structure. Yu et al. (2018) stated that the limited gene flow is due to geographical barriers, local geological history, and environmental stress.

This study revealed genetic diversity, genetic distance, population structure, and genetic flow between giant clam *T. crocea* populations. This study differs from previous studies in that there is a further analysis using the Bayesian model to reveal genetic exchanges between the sites that are the object of study. In addition, gen flow data is also useful for conservation management to determine the priority of protected areas based on source and sink population patterns. The results showed that the *T. crocea* population in the Kei Islands was one genetic population, supported by the paired f_{st} analysis which showed no significant genetic difference due to the low value of f_{st}. Similarly, amova's analysis also showed that there was no population structure. The haplotype tissue analysis showed a mixture of *T. crocea* populations in the Kei Islands. Mixing and haplotype connectivity in the *T. Crocea* population indicated population proximity and a low degree of genetic separation as shown by low genetic distance and high gene flow.

The lack of significant genetic differentiation in *T. crocea* populations in the Kei Islands was suspected due to the distance between populations and oceanographic conditions. Palumbi (2003) stated that populations with close distances tend to have genetic proximity than populations with far distances. All populations of *T. crocea* in the Kei Islands have a relatively close distance between populations, which range from 3.12-103.87 km, and open geographical conditions to avoid the inhibition of genetic exchange between populations. When compared to studies that show population structure and limited gene flow, the geographic distance was very close. Meanwhile, The geographic distance of *T. crocea* populations in the Indo-Malaya Archipelago is 4,770-5,773 km (Kochzius and Nuryanto 2008; de Boor et al. 2008), Indo-West Pacific 4,983 km (Hui et al. 2016), Coral Triangle 6,562 km (DeBoer et al. 2014), and the Indo-Australian Archipelago 7,504 (Keyse et al. 2018). The results are similar to a previous study conducted by Saleky et al. (2016) which obtained genetic closeness to the population of the corded turban (*Turbo sparverius*) and the brown dwarf turban (*T. bruneus*) in the Papua Bird's Head Seascape because all populations are close enough and supported by the New Guinea Coastal Current (NGCC). Apart from geographical distance, the influence of oceanography is an important factor in the process of genetic exchange. This occurred due to the genetic spread of *T. crocea* at the planktonic larval stage. Meanwhile, The existence of currents is important because it becomes a medium of gene transporting between populations. Furthermore, The waters of the Kei Islands are influenced by the monsoon wind system (Wirjohamidjojo and Swarinoto 2010). The monsoon wind pattern also affects the surface currents around the waters of the Kei Islands. During the dry season, the water mass moves from the Arafura Waters to the Banda Sea, while it moves from the Banda Sea to the Arafura Waters during the wet season (Wyrtki 1961). With this current pattern, *T. crocea's* planktonic larvae are easily dispersed to all populations in the Kei Islands, which are only between 3.12-103.87 km apart. A previous study by Mohamed et al. (2016) stated that Tridacna pelagic larvae are dispersed to approximately 500 km.

The total genetic diversity of *T. crocea* populations in the Kei Islands was high (0.89-0.97) based on the index developed by Nei (1987). This result was in line with previous studies by Kochzius and Nuryanto (2008) in the Indo-Melayu Archipelago, which obtained genetic diversity index value 0.60-1.00, Neo and Tood (2012) in Singapore waters (0.86 ± 0.041), DeBoer et al. (2014) in the Coral Triangle (0.80-1.00), and Hui et al. (2016) in the West Indo Pacific (0.94), respectively. Moreover, High genetic diversity values are influenced by gene mutations, population size, reproduction, migration/distribution, and natural selection (Hamilton 2009; Chiu et al. 2013). In this study, the high genetic diversity value was due to the appearance of random mating between population and mutation. Meanwhile, All Tridacna species experienced a planktotrophic stage in their life cycle before becoming benthic species (Lucas 1988) and their larval dispersal spends approximately 9-19 days (Jameson 1976; Mies and Sumida 2012; Triandiza and Kusnadi 2013; Mohamed et al. 2016) drifting with the water current before settling on a hard substrate. This planktonic stage has the potential of

distributing to distant populations and carrying out genetic mixing between populations through random breeding. Furthermore, the high genetic diversity in this study was caused by a mutation that changes in nucleotide sequences which produce genetic diversity. This study discovered 52 polymorphic sites which consist of 29 parsimony and 23 singelton sites. From the 52 locations that had the mutation, 88.6% were transitional substitutes, while 11.54% were transverse substitutions. Based on the Haplotype tissue analysis (Figure 3), each haplotype in the *T. crocea* population was separated by 1-5 mutations.

Dullah and Dar populations had the highest genetic diversity on the molecular marker of mtDNA fragments of COI, while Tanimbar Kei had the lowest. The genetic variation is closely related to the number of haplotypes in a population as shown in Table 1. Generally, the frequency and proportion of haplotypes that occurred in each population showed a fairly even distribution. The Dullah Laut was a population with the most varied habitat diversity, which is characterized by other clam species with approximately 6 species, namely *T. gigas, T. derasa, T. squamosa, T. maxima, T. crocea, and H. hippopus* (Hernawan 2010; Triandiza et al. 2019). This condition is assumed to be one of the important factors for the highest genetic diversity in this region. Moreover, the lowest genetic diversity of Tanimbar Kei is due to its remote location, which is isolated from other islands for more inbreeding.

Although genetic distance and molecular variance analysis showed a genetically mixed population, an asymmetric gene flow between sites was also discovered. Some sites served as source population (Dullah), while others tend to act as a gene flow receiver or sink population such as Dar. Meanwhile, the differences in gene flow at each population in the Kei Islands were due to ocean currents, tides, and habitat selection patterns. Previous studies have shown that the surface current patterns and circulation act as barriers that disrupt gene flow (Ravago-Gotanco and Juinio-Meñez 2010; DeBoer et al. 2014; Treml et al. 2015; Hui et al. 2016). Furthermore, Kuriiwa et al. (2014) stated that the Kuroshio current acts as a barrier to the spread of several marine organisms, and the Kei Islands are influenced by the northwest and southeast monsoons which occur periodically. Also, seasonal changes have an impact on current movement that indirectly influences larval dispersal. Despite the ocean current patterns, gene flow in the Kei Islands was influenced by the tidal phenomenon. This showed that the tides in Kei Islands have a mixed tidal type which occurs twice a day, namely 2 times of high and neap tides, respectively. Moreover, from October to November there was a phenomenon of a very low tide of less than one meter depth that makes the shore dry up until it juts into the middle of the sea, known as *meti kei*. This phenomenon has the potential to inhibit gene flow because not all larvae and juveniles can survive in the dried land. Furthermore, gene flow was influenced by habitat selection where the condition of the micro-habitat becomes an important factor for the distribution of larvae (Albaina et al. 2012). However, not all larvae that arrived at the location are likely to survive, only certain individuals that match the characteristics of the habitat can survive.

Giant clams play an important ecological role on coral reefs and are a source of income and food for the coastal communities. However, the results showed that all clams species are locally threatened with extinction based on population size in nature is less than 1 individual per square meter (Hasni et al. 2017; Ode 2017; Wakum et al. 2017; Harahap et al. 2018; Rizkifar et al. 2019; Triandiza et al. 2019). In this study, genetic data were used to identify the appropriate management strategies for giant clam species. The results showed that there were no genetic differences in the *T. crocea* population in Kei Islands, or it was a single genetic population. These conditions made the management and conservation of giant clam easier since only one conservation management strategy was needed and no various administration at different locations. Meanwhile, the strategy involved the application of natural resource conservation in form of local wisdom, namely S*asi*. Moreover, *Sasi* is the management and protection of natural resources on land and sea which is carried out by the Maluku indigenous people (Ummanah 2013). The tradition of *Sasi* is regulated when the fisherman are allowed to harvest a natural resource with certain limits. Meanwhile, the practice of S*asi* can help restore giant clam populations in all study sites by limiting the harvest time to a certain period. Therefore, the application of *Sasi* for 5 years is recommended because giant clams usually take a certain time to reach adulthood (Bacvard 1981; Fitt 1991).

In conclusion, there was a high level of genetic diversity among *T. crocea* populations in the Kei Islands. The low genetic difference value between populations suggests that the *T. crocea* population on Kei Islands is a single population. The presence of mixed haplotypes indicates gene flow between *T. crocea* populations on Kei Islands. Further gene flow analysis using Bayesian models demonstrated higher genetic exchange between sites, with the Dullah Population primarily serving as a source site for the other sites. The information on genetic diversity is used for aquaculture management, especially on the quality of broodstock selection which is based on high genetic variation (nucleotide) in the population. Similarly, the genetic relationship also needs to be considered on brood-stock selection for the hatchery. A previous study by Suparyanto et al. (1999) stated that inter-population breeding can produce high-quality juveniles with higher heterocyst when using parents with a far genetic distance compared to seeds from parents with a close genetic distance. Furthermore, data on gene flow are also useful for conservation management to determine a specific location for re-stocking and sanctuary. This is based on the pattern of source and sink population, where a location that functions as a source is designed as a priority for protected areas. Based on these results, the most suitable site for marine protected areas and re-stocking site was Dullah Laut due to its high genetic diversity and source population. In addition, Dullah water has a good variety of habitats as indicated by the results of 6 species of giant clam in this area (Hernawan 2010; Triandiza et al. 2019). For aquaculture activities, the parentage of *T. crocea* from

Dullah and Tanimbar Kei was recommended to be used as brood-stock because of the little genetic differences compared to other locations. Furthermore, scientific approaches to the community based on the ecological benefits of clams and the consequences of over-exploitation are conservation management strategies that can be used to prevent the extinction of the clam population in the Kei Islands.

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