Genetic Profile Assessment of Giant Clam Genus Tridacna as a Basis for Resource Management at Wakatobi National Park Waters

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Abstract

Giant clam population has been decreased in a few years. Resource management requires information from various aspects, such as ecological, population, and other aspects. This study was aimed at assessing the genetic profile of Tridacna giant clam in Wakatobi National Park waters using Cytochrome oxidase subunit I (COI) genetic marker. Sample collection was conducted around the three main islands, i.e., Wangi-wangi, Kaledupa, and Tomia. Genetic analysis using COI gene may contribute in identifying giant clams up to the species level and showed the relationship among species. The research found 41 specific nucleotide sites for the clams. T. crocea, T. squamosa and T. maxima had 2, 15 and 24 sites, respectively. COI gene as a biological marker was able to separate groups of giant clam by species. Nucleotide variation of T. crocea from Wakatobi was the highest among other locations, so it could be used as a genetic source for translocation and domestication.

Keywords: *cytochrome oxidase subunit I, specific nucleotide, Tridacna, Wakatobi National Park*

Introduction

Giant clam is one of the bivalve molluscs (Cardiidae, Tridacninae) inhabiting coral reefs and its surroundings. It is attached to coral reefs, as well as burried on sandy substrate on the reefs and seagrass beds (Knop, 1996). Giant clam population has been decreased. Fishing is the main factor of the decline of the wild giant clam population (Shau-Hwai and Yasin, 2003; Romimohtarto and Juwana, 2005; Larrue, 2006). Their commercial appeal encourage the intensively use of clam (shell and meat) by fishermen; therefore, exceeding the carrying capacity of the population in the wild (Panggabean, 1991). Because of the decreased population and the critical need to maintain their sustainability, they had been included in the list of protected biota based on Government Regulation (PP) No. 7 of 1999.

Seven out of eleven species of giant clams of the world inhabit Indonesian waters. Those seven species belong to of two generas, i.e., *Tridacna* and *Hippopus*. There are five species of Tridacna, i.e., *Tridacna gigas, T. derasa, T. squamosa, T. maxima, T. crocea;* while *Hippopus* consists two species, i.e., *Hippopus hippopus* and *H. porcellanus* (Mudjiono, 1988; bin Othman *et al.,* 2010; Hernawan, 2012). Other species that are not found in Indonesia are *T. tevoroa* (Lucas *et al.*, 1990), *T. rosewateri* (Sirenko and Scarlato, 1991), *T. costata* (Richter *et al.*, 2008) and *T. ningaloo* (Penny and Willan, 2014).

Genus *Tridacna* is generally facilitated with mantle, with attractive and flashy colors over the edge of shell (Calumpong, 1992). Their outer shell shape can be divided into two groups, with shell scales, i.e., *T. squamosa, T. maxima and T. crocea* and groups with a shell without scales, i.e., *T. derasa* and *T. gigas* (Knop, 1996). They also have sizes from small to large. The largest size (>100 cm) can be found in this genus, namely *T. gigas.*

One of their distribution areas in Indonesia is Wakatobi National Park waters. Wakatobi National Park is administratively located in Wakatobi of Southeast Sulawesi province. It is defined by the government as a national park by decree of the Minister of Forestry No. 7651/ Kpts-II/2002, and is managed by a zoning system. There are at least five species found there, namely *T. crocea, T. squamosa, T. maxima, T. gigas* and *H. hippopus* (Findra, 2010).

Resource management requires information from various aspects, both biological, population and

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others, so it would be more focused and successful. The genetic information is one of the aspect that is needed in the management and conservation. In Indonesia, it is still less noticeable, whereas the role of adaptation and animal development strategy is largely determined by genetic capabilities. Genetic diversity information can be obtained by analyzing the protein-coding genes of mitochondrial DNA. Part of them often used in species study and animal population is Cytochrome oxidase subunit I (COI) (Solihin, 1994).

COI is a gene that evolved very slowly so it can be used as DNA barcoding (Hebert *et al.,* 2003). It is an efficient method for the spesies identification and has a role in biodiversity taxonomic and population genetics study (Hajibabaei *et al.,* 2007). Studies using COI genes as genetic markers of the giant clams have been conducted few years ago by Nuryanto *et al*. (2007) in several places in Indonesia, Tisera *et al*. (2012) in Savu Sea East Nusa Tenggara, Lizano and Santos (2014) in the Philippines. However, giant clam population of Wakatobi waters has not been genetically identified. Therefore, this study was aimed at assessing the genetic profile of giant clams using COI genetic markers especially genus *Tridacna* in the Wakatobi National Park as a data for resource management.

Materials and Methods

Giant clams were collected from Wakatobi National Park waters around the three main islands, i.e., Wangi-wangi, Kaledupa and Tomia (Figure 1.). Analysis of samples carried out in Laboratory of Animal Biomolecular, Research Center for Biological Resources and Biotechnology (PPSHB) and the Integrated Laboratory of the Department of Biology, Bogor Agricultural University.

Sample of each species identified based on the description by Knop (1996) was collected from each sampling location. Samples were taken from mantle tissue using scissors. Samples were inserted into the tube and then preserved using 96% alcohol. (Table 1).

Total DNA Isolation and Extraction

Prior to isolation and extraction total DNA, samples were washed using Low TE so that the sample free of alcohol as a preservative. Further samples were isolated using a commercial kit from GeneAid. Its procedures performed following the manual from the factory with some procedures that had been accordingly modified.

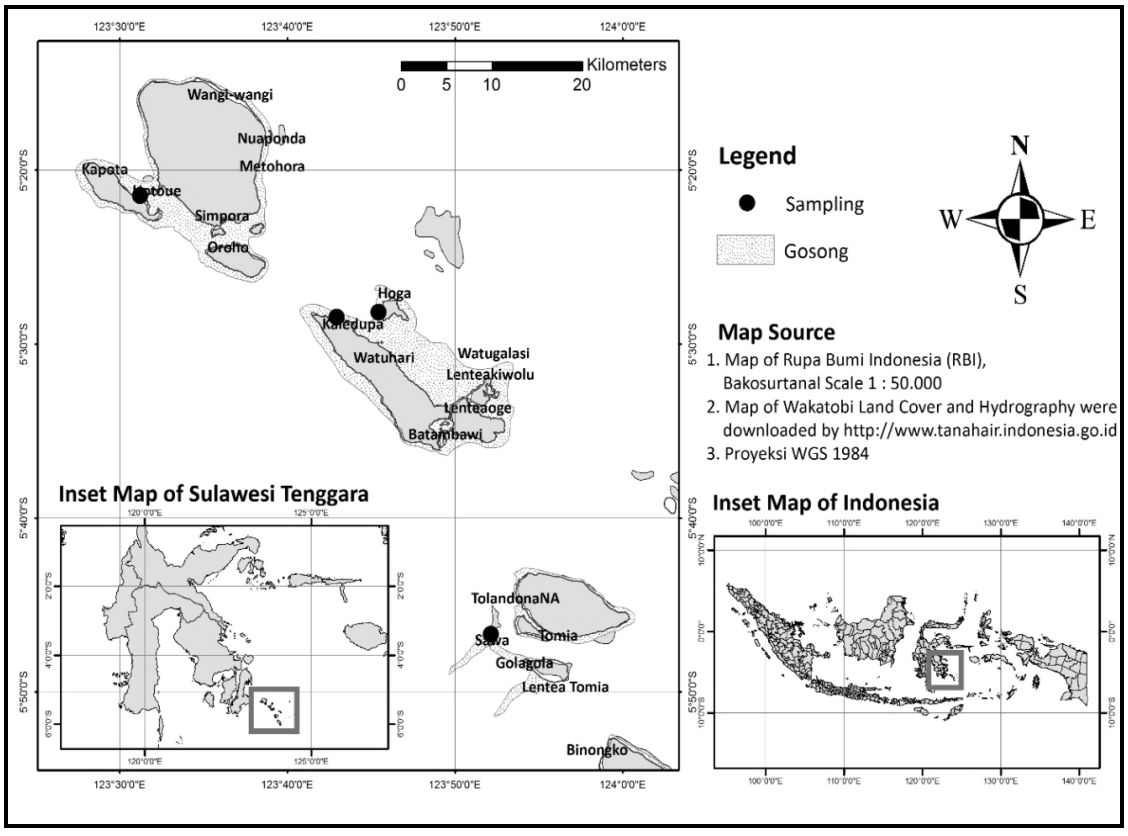

Figure 1. Map of sampling locations at Wakatobi National Park, Sulawesi.

Target Gene Amplification, Sequencing and Data Analysis

Target gene segment was amplified using PCR (Polymerase Chain Reaction). Amplification used specific primers for tridacnid that had been designed by Nuryanto *et al.* (2007), LCO: 5'-GGG GAA TTC TAA TGA CAG AA-3 'and RCO: 5'-TAG TTA CAG CTA CCC AAG AA-3'. The reaction total volume was 25 ml consisting of 9.8 ml of ddH_2O, 4 ml Q5 buffer, Q5 enhancer 5 ml, 1 ml of dNTP, 1 µl forwards primer, reverse primer 1 ml, 3 ml of DNA template and 0.2 ml of Q5 Taq Hot Start.

Amplification was conducted under predenaturation 95°C for 5 minutes, followed by 35 cycles consisting of denaturation 94°C for 45 seconds; annealing 49°C for *T. crocea* and *T. maxima*, and 52°C for *T. squamosa* for 45 seconds; and extension 72°C for 1 minute and final extension of 72°C for 7 minutes. The amplicons were tested electrophoresis using 1.2% agarose gel in 1X TBE (Tris-borate-EDTA) buffer. PCR products were either single band seen during electrophoresis (size 522 bp) proceed to the stage of sequencing to look at the sequence of nucleotide. They were sent to 1st BASE Sequencing, Malaysia.

Sequences were corrected and aligned using software MEGA 5.0 (Tamura *et al.*, 2011). Sequences from each sample was BLAST in GenBank to determine the proximity to other sequences stored in GenBank. The closeness became secondary sequence data to be analyzed to produce phylogenetic tree. Its reconstruction used Neighbour Joining method with p-distance model, 1000 bootstrap replicates. These analysis used several sequences from GenBank as their ingroup and outgroup. They were *T. crocea* from Spermonde Islands (Accession EU003606), *T. crocea* from Seribu Island (Accession EU003608), *T. maxima* from Padang (Accession EU003610), *T. maxima* from Biak (Accession EU003613), *T. squamosa* from Philippines (Accession KJ202117) and *H. hippopus* from Philippines (Accession KJ202106).

Results and Discussion

There were seven successfully amplified sequences (from 24 samples) using specific primers, while the others were not successfully amplified. It could be due to unsuccess DNA extraction process. The failure was thought to be caused by the presence of impurities in the form of residual preservatives and mucus, as well as their algal symbionts zooxanthellae contained in the mantle tissue of clams. The similar case was reported by Haerul (2014), that symbionts algae became impurities in DNA extraction process.

The sequences were successfully traced the nucleotides derived from three species, i.e., *T. crocea, T. squamosa* and *T. maxima*. The forward and reverse sequences of each individual were combined and aligned, so we obtained the nucleotide size of ±522 bp (Figure 2).

Several samples that morphologically identified as *T. squamosa,* following validation using BLAST that those performed different species (Table 2). TsH 1 and TsS 1 were originally identified as *T. squamosa* after BLAST indicated that they were *T. crocea* and *T. maxima*, respectively. In aquatic organism, we often found cryptic species phenomenon, which was morphologically similar, however; it was different in genetics. It could lead to false identification (Bickford *et al.*, 2006). This study showed that giant clams were cryptic species, their morphologies were alike among distinct species, so often misidentified by their morphology. These three are included in a group which have scales and their shells are generally embedded in part or whole in

Table 1. Species and number of samples collection

No.	Species	Location	Code	Number
1.	*T. crocea*	Langgira, Kaledupa	TcL	2
		Hoga Island, Kaledupa	TcH	3
		Sawa Island, Tomia	TcS	1
2.	*T. squamosa*	Kapota Island, Wangi-wangi	TsW	1
		Langgira, Kaledupa	TsL	5
		Hoga Island, Kaledupa	TsH	5
		Sawa Island, Tomia	TsS	1
3.	*T. maxima*	Langgira, Kaledupa	TmL	1
		Hoga Island, Kaledupa	TmH	3
		Sawa Island, Tomia	TmS	1
	Total			24

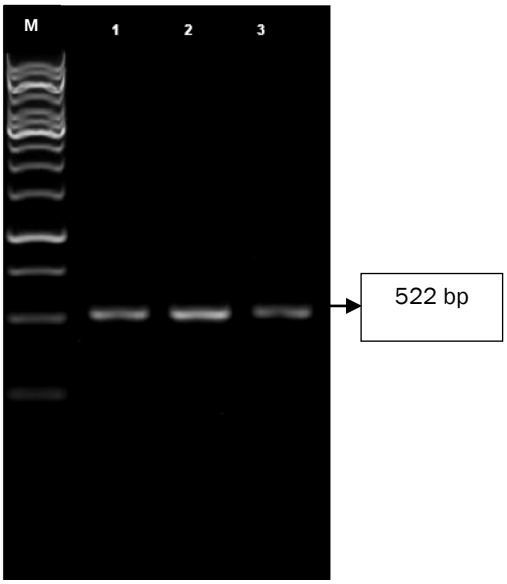

Figure 2. PCR products successfully amplified using COI gene showing intact bands (M = 1 kb DNA Ladder; 1 = *T. crocea*; 2 = *T. squamosa*; 3 = *T. maxima*)

the reef, so only mantle was clearly shown up. In addition, samples taken in this study were still juvenile, so it was difficult to identify up to species level. This study also proved that molecular analysis using COI genetic markers may contribute in identifying giant clams up to the species level.

Alignment of seven COI gene sequences consisting of four, one and two sequences *T. crocea, T. squamosa* and *T. maxima*, respectively, showed that few variable sites (17.82%), while conserve sites as many as 82.18%. According to Herbert *et al.* (2003), COI gene has conservative nucleotide base composition with a bit insertions, deletions and variations so that is used as DNA barcoding.

This research found 41 specific nucleotide sites, *T. crocea, T. squamosa* and *T. maxima* had 2, 15, and 24 sites, respectively (Table 3). Those nucleotide sites were the specific genetic marker which can differentiate those species.

The genetic distance intra-species was less than 2% and inter-species was more than 5% (Table 4). *T. crocea, T. squamosa* and *T. maxima* had genetic distance of 1.43%, 0.93%, and 1.40%, respectively. Genetic distance of less than or equal to 3% could be said similar species, while the genetic distance of more than 3% showed different species. According to Ratnasingham and Hebert (2013), COI gene variations of more than 4% are close relatives and isolated reproduction, if the difference of less than 2% are the same species (intra-species).

The phylogenetic tree showed that generally formed two clades (Figure 3): the first consisted of *T. crocea, T. squamosa* and *T. maxima,* the second clade was *H. hippopus*. It indicated that all species of the genus *Tridacna* were monophyletic and separated from *H. hippopus* which was another genus. Phylogenetic tree reconstructed by Nuryanto *et al.* (2007) using the Neighbour Joining method also showed the same phenomenon, that all species of the genus *Tridacna* was monophyletic. *T. crocea* and *T. squamosa* were in the same subclade, while T. *maxima* and *T. gigas* were in other subclade. Phylogenetic tree reconstructed by Lizano and Santos (2014) showed the same trend, *T. crocea* and *T. squamosa* were also at the same subclade. But, there was a difference between Nuryanto *et al.* (2007) and Lizano and Santos (2014). Phylogenetic tree reconstructed by Nuryanto *et al.* (2007) showed that *T. gigas* and *T. maxima* were in one group and sister taxa with group of *T. crocea* and *T. squamosa*, whereas phylogenetic tree reconstructed by Lizano and Santos (2014) showed that *T. gigas* separately clustered from group of *T. crocea, T. squamosa* and *T. maxima*. Reconstruction of phylogenetic tree using 16S rRNA gene fragment by Schneider and O'Foighil (1999) also showed that all species of the genus *Tridacna* were monophyletic, as well as the genus *Hippopus*. Tridacna clade formed two groups, the first group consisted of *T. tevoroa* (*T. gigas* + *T. derasa*), and the second group consisted of *T. maxima* (*T. squamosa* + *T. crocea*). Reconstruction of the phylogenetic tree using either COI or 16S rRNA gene fragment showed consistent results for *T. crocea, T. squamosa* and *T. maxima*. They were

Table 2. Results of nucleotide bases BLAST in GenBank

No.	Sample	Query Cover	Identity	Species Validation	Accession
1	TcL 1	100%	99%	*T. crocea*	DQ269479.1
2	TcH 2	100%	98%	*T. crocea*	DQ269479.1
3	TcS 1	100%	98%	*T. crocea*	DQ269479.1
4	TsW 1	98%	97%	*T. squamosa*	KP205428.1
5	TsH 1	100%	98%	*T. crocea*	DQ269479.1
6	TsS 1	97%	99%	*T. maxima*	DQ155301.2
7	TmH 1	98%	99%	*T. maxima*	DQ155301.2

Table 3. Specific nucleotides of giant clam species

Species	Nucleotide position													
	9	16	18	28	29	30	36	42	45	87	90	105	108	150
T. crocea Wakatobi 1	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. crocea Wakatobi 2	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. crocea Wakatobi 3	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. crocea Wakatobi 4	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. crocea Spermonde GB*	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. crocea Seribu Island GB*	T	T	G	G	C	C	T	A	T	A	A	T	T	A
T. squamosa Wakatobi	C	T	A	G	C	C	C	G	T	A	A	C	C	A
T. squamosa Filipina GB*	C	T	A	G	C	C	C	G	T	A	A	C	C	A
T. maxima Wakatobi 1	T	C	G	A	G	A	T	A	C	G	G	T	T	G
T. maxima Wakatobi 2	T	C	G	A	G	A	T	A	C	G	G	T	T	G
T. maxima Padang GB*	T	C	G	A	G	A	T	A	C	G	G	T	T	G
T. maxima Biak GB*	T	C	G	A	G	A	T	A	C	G	G	T	T	G

Species	Nucleotide position													
	156	168	172	174	189	192	205	208	216	222	238	249	258	268
T. crocea Wakatobi 1	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. crocea Wakatobi 2	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. crocea Wakatobi 3	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. crocea Wakatobi 4	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. crocea Spermonde GB*	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. crocea Seribu Island GB*	C	C	C	A	G	A	T	C	G	G	G	T	C	T
T. squamosa Wakatobi	T	C	C	A	G	A	G	C	G	G	G	C	T	T
T. squamosa Filipina GB*	T	C	C	A	G	A	G	C	G	G	G	C	T	T
T. maxima Wakatobi 1	C	T	T	G	A	T	T	T	A	A	A	T	C	C
T. maxima Wakatobi 2	C	T	T	G	A	T	T	T	A	A	A	T	C	C
T. maxima Padang GB*	C	T	T	G	A	T	T	T	A	A	A	T	C	C
T. maxima Biak GB*	C	T	T	G	A	T	T	T	A	A	A	T	C	C

Species	Nucleotide position												
	270	282	286	297	306	339	342	354	357	360	366	372	417
T. crocea Wakatobi 1	G	T	C	T	T	T	T	T	G	G	C	G	C
T. crocea Wakatobi 2	G	T	C	T	T	T	T	T	G	G	C	G	C
T. crocea Wakatobi 3	G	T	C	T	T	T	T	T	G	A	T	G	C
T. crocea Wakatobi 4	G	T	C	T	T	T	T	T	G	G	C	G	C
T. crocea Spermonde GB*	G	T	C	T	T	T	T	T	G	A	T	G	C
T. crocea Seribu Island GB*	G	T	C	T	T	T	T	T	G	A	T	G	C
T. squamosa Wakatobi	A	C	C	T	C	T	T	T	A	A	T	A	C
T. squamosa Filipina GB*	A	C	C	T	C	T	T	T	A	A	T	A	C
T. maxima Wakatobi 1	G	T	T	G	T	C	A	C	G	A	T	G	T
T. maxima Wakatobi 2	G	T	T	G	T	C	A	C	G	A	T	G	T
T. maxima Padang GB*	G	T	T	G	T	C	A	C	G	A	T	G	T
T. maxima Biak GB*	G	T	T	G	T	C	A	C	G	A	T	G	T

* GB = GenBank Data

T. crocea Wakatobi 1 from Langgira, Kaledupa; *T. crocea* Wakatobi 2 and 4 from Hoga Island, Kaledupa; *T. crocea* Wakatobi 3 from Sawa Island, Tomia; *T. squamosa* Wakatobi from Kapota Island, Wangi-wangi; *T. maxima* Wakatobi 1 from Hoga Island, Kaledupa; *T. maxima* Wakatobi 2 from Sawa Island, Tomia.

included in subgenus *Chametrachea*. According to Hernawan (2012), genus *Tridacna* consisted of three subgenus, namely *Tridacna sensu strict, Persikima* and *Chametrachea*. Subgenus *Tridacna strict sensu* included only *T. gigas*, subgenus *Persikima* consisted of *T. derasa* and *T. tevoroa*, subgenus *Chametrachea* composed of *T. squamosa, T. crocea, T. maxima, T. costata,* and *T. rosewateri*.

The phylogenetic tree also showed that *T. crocea* formed two groups, the first group consisted of *T. crocea* Spermonde, *T. crocea* Seribu Island and *T. crocea* Wakatobi 3, while the second group consisted of *T. crocea* Wakatobi 1, *T. crocea* Wakatobi 2 and *T. crocea* Wakatobi 4. Wakatobi *T. crocea* was highly varied, because it possesses not only similar nucleotide sequences of Spermonde and Seribu Island *T. crocea* but also specific nucleotide sequences Wakatobi *T. crocea* itself (Table 5). It was due to varying environmental characteristics in these waters. Difference in habitat typology and geographically isolated would lead to

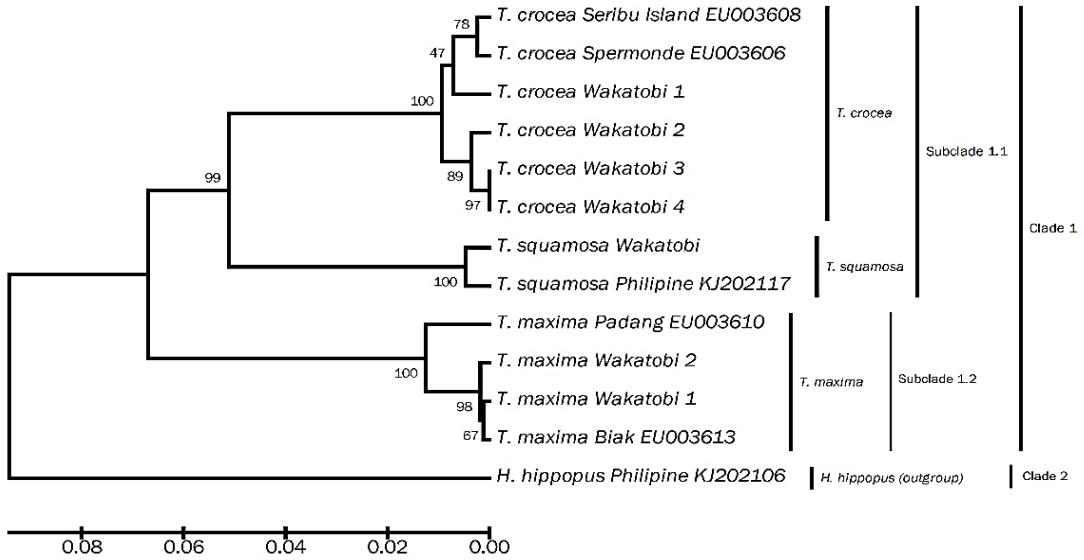

Figure 3. Reconstruction of phylogenetic tree using Neighbour Joining method with p-distance model, 1000 bootstrap replicates.

Table 4. Genetic distance of giant clam COI gene using p-distance model

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0.019												
3	0.021	0.007											
4	0.021	0.007	0.000										
5	0.012	0.016	0.019	0.019									
6	0.016	0.016	0.019	0.019	0.005								
7	0.105	0.109	0.107	0.107	0.100	0.100							
8	0.100	0.105	0.102	0.102	0.095	0.095	0.009						
9	0.133	0.137	0.130	0.130	0.133	0.133	0.142	0.142					
10	0.130	0.135	0.128	0.128	0.130	0.130	0.140	0.140	0.002				
11	0.128	0.133	0.130	0.130	0.128	0.128	0.137	0.137	0.023	0.026			
12	0.135	0.140	0.133	0.133	0.135	0.135	0.144	0.144	0.002	0.005	0.026		
13	0.191	0.191	0.188	0.188	0.195	0.195	0.207	0.207	0.174	0.177	0.177	0.172	0.247

1= *T. crocea* Wakatobi 1; 2 = *T. crocea* Wakatobi 2; 3 = *T. crocea* Wakatobi 3; 4 = *T. crocea* Wakatobi 4; 5 = *T. crocea* Spermonde EU003606; 6 = *T. crocea* P. Seribu EU003608; 7 = *T. squamosa* Wakatobi; 8 = *T. squamosa* Philipine KJ202117; 9 = *T. maxima* Wakatobi 1; 10 = *T. maxima* Wakatobi 2; 11 = *T. maxima* Padang EU003610; 12 = *T. maxima* Biak EU003613; 13 = *H. hippopus* KJ202106 (out group)

Table 5. *T. crocea* nucleotide polymorphism from Wakatobi, Spermonde and Seribu Island

Species	Nucleotide position														
	1	.	.	.	.	333	.	.	360	.	366	.
T. crocea Wakatobi 1	T	.	.	.	.	A	.	.	G	.	C	.
T. crocea Wakatobi 2	T	.	.	.	.	A	.	.	G	.	C	.
T. crocea Wakatobi 4	T	.	.	.	.	A	.	.	G	.	C	.
T. crocea Wakatobi 3	T	.	.	.	.	G	.	.	A	.	T	.
T. crocea Spermonde GB*	T	.	.	.	.	G	.	.	A	.	T	.
T. crocea Seribu Island GB*	T	.	.	.	.	G	.	.	A	.	T	.

*GB = GenBank Data

Table 6. *T. maxima* nucleotide polymorphism from Wakatobi, Biak and Padang

Species	Nucleotide position								
	342	171	234	237	243	285	294	312	327
T. maxima Wakatobi 1	C	T	A	C	T	C	T	C	C
T. maxima Wakatobi 2	C	T	A	C	T	C	T	C	C
T. maxima Biak GB*	C	T	A	C	T	C	T	C	C
T. maxima Padang GB*	T	C	C	T	C	T	C	T	T

*GB = GenBank Data

different genetic structure of bivalve (Donrung *et al*., 2011), even in a long time would result in different morphological forms (Evans and Hoffman, 2012). *T. maxima* also formed two groups, which *T. maxima* from Wakatobi clustered with *T. maxima* from Biak and separated with *T. maxima* from Padang. It was also caused by a similarity in the nucleotide composition between *T. maxima* from Wakatobi and *T. maxima* from Biak (Table 6).

Genetic information could be utilized in giant clams resource management. *T. crocea* Wakatobi varying might be used as a genetic resource. In domestication, it could also be used as a broodstock because it was more genetically varied. Likewise, if we would translocate the clam, we could catch *T. crocea* from Wakatobi as the genetic source. According to Yusron (2005), the strategy to increase the biodiversity of a population and to improve decreasing genetic diversity besides reducing the exploitation rate was introduction of new individuals which had higher genetic diversity into the local population.

Conclusion

There were 41 specific nucleotide sites that became barcode of each species, *T. crocea, T. squamosa* and *T. maxima* had 2, 15, and 24 sites, respectively. Those facilitated uncovering cryptic species phenomenon in this genus. Nucleotide variation of *T. crocea* from Wakatobi was the highest among *T. crocea* from other locations; therefore, it could be used as a genetic source for translocation and domestication.

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References

Bickford, D., Lohman, D.J. , Sodhi, N.S., Ng, P.K.L., Meier, R., Winkler, K., Ingram. K.K., & Das, I. 2006. Cryptic species as a window on diversity and conservation. *Ecol. and Evol..* 22:148-155.

bin Othman, A.S., Goh, G.H.S. & Todd, P.A.. 2010. The distribution and status of giant clams (Family Tridacnidae), a short review. *Raffles Bull Zool.* 58(1):103-111.

Calumpong, H.P. (Ed). 1992. The Giant Clam: An Ocean Culture Manual. ACIAR Monograph. Canberra. 68 pp.

Donrung, P., Tunkijjanukij, S., Jarayabhand, P. & Poompuang, S. 2011. Spatial genetic structure of the surf clam *Paphia undulata* in Thailand Waters. *Zoological Studie*s. 50(2): 211-219.

Evans, T.G. & Hoffman, G.E. 2012. Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Biological Science.* 367: 1733-1745.

Findra, M.N. 2010. Komposisi Jenis, Kelimpahan dan Ukuran Kima di Perairan Pulau Tolandono dan Pulau Sawa, Kawasan Taman Nasional Wakatobi. Skripsi. Universitas Hasanuddin. Makassar. 62 pp.

Haerul, A. 2014. Karakterisasi Genetik Karang Genus Favites (Faviidae: Scleractinia) di Perairan Kepulauan Spermonde, Sulawesi Selatan. Tesis. Institut Pertanian Bogor. Bogor. 64 pp.

Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N., & Hickey, D.A. 2007. DNA barcoding: how it complements taxonomy, molecular

phylogenetics and population genetics. *TRENDS in Genetics.* 23(4):167-172. doi: 10.1016/j.tig. 2007.02.001.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences. 270(1512):313-321.

Hernawan, U.E. 2012. Taxonomy of Indonesian giant clams (Cardiidae, Tridacninae). *Biodiversitas.* 13(3):118-123.

Knop, D. 1996. Giant Clams: A Comprehensive Guide to the Identification and Care of Tridacnid Clams. Dahne Verlag. Germany. 255 pp.

Lizano, A.M.D. & M.D. Santos. 2014. Updates on the status of giant clams *Tridacna* spp. and *Hippopus hippopus* in the Philippines using mitochondrial CO1 and 16S rRNA genes. *Philippine Science Letters*. 7(1):187-199.

Larrue, S. 2006. Giant clam fishing on the Island of Tubuai, Austral Islands group: between local portrayals, economic necessity and ecological realities. *SPC Traditional Marine Resource Management and Knowledge Information Bulletin.* (19):3-40.

Lucas, J.S., Ledua, E. & Braley, R.D. 1990. A new species of giant clam (Tridacnidae) from Fiji and Tonga. Australian Centre for International Agricultural Research. *Working Paper.* 33:1-8.

Mudjiono. 1988. Catatan beberapa aspek kehidupan kima, Suku Tridacnidae. *Oseana.* 13(2):37-47.

Nuryanto, A., Duryadi, D., Soedharma, D., & Blohm, D. 2007. Molecular phylogeny of giant clam based on mitochondrial DNA Cytochrome C Oxidase I Gene. *Hayati J. Biosci.* 14(4):162-166.

Panggabean, L.M.G. 1991. Rahasia kehidupan kima: III. Kelangsungan hidup. *Oseana.* 16(2):35-45.

Penny, S.S. & Willan, R.C. 2014. Description of a new species of giant clam (Bivalvia: Tridacnidae) from Ningaloo Reef, Western Australia. *Mollus. Res.* 34:201–211.

Ratnasingham, S. & Hebert, P.D.N. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. *Plos One*. 8(8):e66213.

Richter, C., Roa-Quiaoit, H., Jantzen, C., Al-Zibdah, M. & Kochzius, M. 2008. Collapse of a new living species of giant clam in the Red Sea. *Curr. Biol*. 18:1349–1354.

Romimohtarto, K. &. Juwana, S., 2005. Biologi Laut. Djambatan. Jakarta. 540 pp.

Shau-Hwai A.T. & Z. Yasin. 2003. Status of giant clams in Malaysia. *SPC Trochus Information Bulletin.* 10:9-10.

Sirenko, B.I. & Scarlato, O.A.. 1991. *Tridacna rosewateri sp.n.* A new species of giant clam from the Indian Ocean. *La Conchiglia*. 22(261):4-9.

Schneider, J.A. & O´Foighil, D.1999. Phylogeny of giant clams (cardiidae: tridacninae) based on partial mitochondrial 16S rDNA gene sequences. *Mol. Phylog. Evol*. 13(1):59–66.

Solihin, D.D. 1994. Peran DNA mitokondria (mtDNA) dalam studi keragaman genetik dan biologi populasi pada hewan. *Hayati.* 1(1):1-4.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28(10):2731-2739.

Tisera, W.I., Naguit, M.R.A. Rehatta, B.M. & Calumpong, H.P. 2012. Ecology and genetic structure of giant clams around Savu Sea, East Nusa Tenggara Province, Indonesia. *Asian J. Biodiversity*. 10:174-194.

Yusron, E. 2005. Pemanfaatan keragaman genetik dalam pengelolaan sumberdaya hayati laut. *Oseana*, 30(2):29-34.