Genetic Variability in the Indonesian Giant Clam (*Tridacna crocea* and *Tridacna maxima*) Populations: Implication for Mariculture and Restocking Program

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Abstract

Tridacna crocea *and* T. maxima *are relatively abundant in the Indonesian coral reef. These two species are, however, under high presure due to exploitation for food, industry, and aquarium trade. It is, therefore, necessary to understand their biology, such as genetic variability within and between populations, before utilizing them for strain improvement and restocking, prior to the extinction of the populations of T. crocea* and *T. maxima*. *Here we amplified a length of 456 bp of the mitochondrial DNA cytochrome c oxidase I gene from* Tridacna crocea *and of 484 bp from* T. maxima *to asses the genetic variability within and between populations of both species. The results showed that both species have high genetic diversity and polymorphism within each local population. This provides a sufficient basis for selection of improved strain of* T. crocea *and* T. maxima *for mariculture. However, if the genetic variation led to genetic differentiation among populations due to the result of evolutionary adaptation, mixing genetically different populations may result in the break up of co-adaptation gene complexes. This might result in the loss of the physiological capacities of the parental populations.*

Key words: *genetic variability, cytochrome c oxidase I gene,* Tridacna crocea, Tridacna maxima

Introduction

The family of tridacnidae, also known as giant clam, is conspicuous bivalve that inhabits coral reef across the Indo-Pacific region (Lucas, 1988). There are eight extant species recognized, seven of which are found in Indonesia, i.e. *Hippopus hippopus, H. porcellanus, Tridacna gigas* Linnaeus 1758, *T. maxima* Röding 1798, *T. squamosa* Lamarck 1819, *T. crocea* Lamarck 1819, and *T. derasa* Röding 1798 (Lucas *et al*., 1991).

All species of giant clam are important fisheries commodities in Indonesia, either as food resources, raw material for various industries (e.g. ceramics and handicraft industries) or for aquarium trade (Pasaribu, 1988; Wells, 1997). This condition led them strongly harvested and are listed as vulnerable species in the CITES Red List Data Book (Wells, 1997) due to over-exploitation (Lucas, 1988).

Indonesian giant clam are fully protected since 1987 based on the Ministerial decree No. 12/Kpts-II, 12 January 1987. According to this law, harvesting and sale of giant clam are strongly prohibited, except with special permit (Pasaribu, 1988). However, collecting and illegal trading has been continued and that Ministerial decree has to be difficult to enforce.

T. crocea and *T. maxima* are two species still relatively abundant in the Indonesian coral reefs, whereas the remaining species are rare if not endangered (personal observation). It is, therefore, necessary to understand their biology, such as genetic variability within and between populations, before utilizing them for strain improvement and restocking, prior to the extinction of both populations.

Previous studies described the high level of genetic variability in *T. maxima* within single populations collected in each of the Marshal Island and the Great Barrier Reef

(GBR) (Ayala *et al*., 1973; Campbell *et al*., 1975). High degree of genetic variation was also reported in *T. gigas*, *T. derasa* (Macaranas *et al*., 1992) and *T. crocea* (Juinio-Menez *et al*., 2003). These studies were outside the Indonesian region and based on protein electrophoresis data. However, there is at present no data on genetic variation of *T. crocea* and *T. maxima* in Indonesia, either using protein electrophoresis or DNA-based data.

Genetic variations of populations are affected by their breeding system, population size, and their recent histories. The character has significant effect on local adaptations, population fitness (Kenchington and Heino, 2003; Meffe and Carrol, 1994) and energy metabolism (Koehn, 1991). This implies that selection for good production traits like growth rate should necessarily maintain some degree of variability. Mixing the genetically diverse stock would enhance genetic variation and assist the production of improved strains for mariculture (Macaranas *et al*., 1992).

Here we studied genetic variability of the boring giant clam (*T. crocea*) and rugose clam (*T. maxima*) using partial sequences of the mitochondrial DNA cytochrome c oxidase I (COI) gene. This gene was chosen due to their high mutation rate, lack of recombination (Hebert *et al*., 2003a), highly divergent among populations (Bucklin *et al*., 2003), and broader phylogenetic sign (Hebert *et al*., 2003b). Therefore, it is suggested that fragment of COI gene is suitable for studying genetic variation within species. This study was aimed to provide genetic data on *T. crocea* and *T. maxima* populations in Indonesia to facilitate the development of mariculture and restocking program.

Material and Methods

Mantle tissues were collected at several locations in the Indonesian Archipelago during the field trips in 2004 and 2005. A small piece of mantel tissues was cut off from each specimen with the help of scissors and forceps. Tissue samples were preserved on 96% of ethanol and stored at 4°C. Total genomic DNA was isolated using Chelex® method following the protocols from Walsh *et al*. (1991).

A fragment of cytochrome c oxidase I (COI) gene was amplified using a pair of *T. crocea* specific primer (COI-Tricro-Frwd: 5'-GGG TGA TAA TTC GAA CAG AA-3' and COI-Tricro-Rev: 5'-TAG TTA AGG CCC CAG CTA AA-3'). PCR reactions were carried out in a total volume of 50 µl containing approximately 10 picogram of DNA template, 1 x PCR buffer, 2 mM of $MgCl_2$, 0.02 µM of each primer, 0.2 mM of each dNTPs, and 1 unit Taq polymerase. Thermal cycles were as follows: one cycle at 94°C for 5 minutes, followed by 35 cycles of 1 minute at 94°C, 1.5 minutes annealing temperature (at 50°C for *T. maxima* and 43°C for *T. crocea* respectively), and 1 minute at 72°C for extension. Final extension was carried out at 72°C for five minutes.

The PCR products were purified using the QIA quick spin column PCR purification kit (Qiagen, Hilden, Germany) following the protocol from the manufacturer. Both strands were sequenced using the DyeDeoxy terminator chemistry (PE Biosystem, Foster City) and an automated sequencer (ABI prism 310; Applied Biosystem, Weiterstadt).

All sequences were initially aligned and edited manually using Sequences Navigator (version 1.0.1; Applied Biosystem). Multiple sequences alignment was done using ClustalW (Thompson *et al.*, 1994) as implemented in Bioedit version 7.0.4.1 (Hall, 1999). Genetic diversity was estimated as haplotype diversity *h* (Nei, 1987) and nucleotide diversity π (Nei and Jin, 1989) was calculated with the programme Arlequin (ver. 2.0; Schneider *et al*., 2000).

Results and Discussion

Sequences similarity with BLAST showed that the obtained sequences are homologous to *Tridacna crocea* sequences which are collected in Japan (accession number AB76920). The obtained sequences are functional fragment of the cytochrome c oxidase I (COI) gene. This was verified by translating all nucleotide sequences to amino acid sequences and there was no stop codon observed along the sequences.

A multiple aligment resulted in a length of 456 bp and 484 bp fragments of the mitochondrial DNA COI gene for *T. crocea* and *T. maxima* respectively. A total number of 300 sequences (individuals) were obtained for *T. crocea* which were collected at 15 localities across the Indonesian Archipelago and of 211 sequences for *T. maxima* that are collected at 14 sites in the Indonesian Archipelago and the Red Sea.

Among 300 individuals of *T. crocea*, 132 haplotypes were obtained. From 456 bp of the COI gene, 117 polymorphic sites (25.7%) were observed indicating high polymorphism. For *T. maxima*, of 211 individuals there were 117 haplotypes obtained and among 484 bp fragment a total of 116 (23.97%) polymorphic sites were observed, also indicating high polymorphism.

For both species, genetic diversity analysis within each population showed high level of haplotype and nucleotide diversity. However, there was a complex pattern of genetic diversity within certain population when we compared both species. For examples, *T. crocea* showed low level genetic diversity for both populations from the Java Sea (Karimunjava and Pulau Seribu), while in the case of *T. maxima* only the population from Karimunjava showed low degree genetic diversity. A similar pattern was also observed in the population from Sulawesi. Genetic diversity of *T. crocea* population from Togian Islands was low while that of *T. maxima* population from Spermonde was low. The *T. crocea* population from Komodo showed high degree of genetic diversity but it is not the case for that of *T. maxima*. Haplotype diversity of *T. crocea* ranged from 0.60 in the population from Pulau Seribu to 1.00 in that from Manado showing nucleotide diversity from 0.68% in Pulau Seribu to 4.34% in Biak (Table 1). Overall haplotype diversity (*h*) of *T. maxima* was 0.93 and nucleotide diversity (π) was 1.5 %. Haplotype diversity of *T. maxima* ranged from 0.59 in the population from Karimunjava to 1.00 in Bira showing nucleotide diversity from 0.24% in Komodo to 4.51% in Biak (Table 1). Overall haplotype diversity (*h*) was 0.94 and nucleotide diversity (π) was 3.2 %.

Table 1. Sample sites, haplotype and nucleotide diversities of *T. maxima* and *T. crocea*

Code	Sample site	*Tridacna maxima*		*Tridacna crocea*	
		h	π (%)	*h*	π (%)
RS	Red Sea	0.95 ± 0.051	0.59 ± 0.37	-	-
Pa	Padang	0.92 ± 0.053	0.53 ± 0.34	0.95 ± 0.096	1.03 ± 0.66
PS	Pulau Seribu	0.91 ± 0.080	0.32 ± 0.23	**0.60 ± 0.150**	0.68 ± 0.42
Ka	Karimunjava	**0.59 ± 0.130**	0.60 ± 0.37	**0.77 ± 0.113**	0.69 ± 0.42
KK	Kota Kinabalu	-	-	0.97 ± 0.023	1.14 ± 0.64
Sa	Sangalaki	0.86 ± 0.137	0.84 ± 0.54	0.97 ± 0.036	1.31 ± 0.74
Sp	Spermonde	**0.69 ± 0.115**	0.56 ± 0.34	0.96 ± 0.014	1.05 ± 0.58
Bi	Bira	1.00 ± 0.045	0.76 ± 0.48	0.91 ± 0.065	0.89 ± 0.54
Se	Sembilan	0.85 ± 0.104	0.46 ± 0.31	0.94 ± 0.043	1.28 ± 0.72
Ke	Kendari	-	-	0.83 ± 0.074	0.79 ± 0.46
Lu	Luwuk	0.90 ± 0.062	0.45 ± 0.30	0.82 ± 0.082	0.73 ± 0.44
TI	Togian Islands	0.99 ± 0.019	0.86 ± 0.50	**0.79 ± 0.063**	0.71 ± 0.41
Ma	Manado	0.90 ± 0.057	0.43 ± 0.28	1.00 ± 0.063	0.85 ± 0.54
Ko	Komodo	**0.77 ± 0.128**	0.24 ± 0.19	0.89 ± 0.059	1.01 ± 0.57
Ku	Kupang	0.89 ± 0.081	0.81 ± 0.49	0.92 ± 0.092	0.70 ± 0.45
Bk	Biak	0.98 ± 0.028	4.51 ± 2.35	0.99 ± 0.031	4.34 ± 2.29
Overall		0.94	3.2%	0.93	1.5%

Both species of giant clams showed high level of genetic diversity and polymorphism. This provides a sufficient basis for selection of improved strain of *T. crocea* and *T. maxima* for mariculture. The rational is that high genetic diversity provides wide range of adaptive character to the environment and population fitness (Kenchington and Heino, 2003; Meffe and Carrol, 1994), reproductive strategy and growth rate of the species through enhancing energy metabolism (Koehn, 1991). Campbell *et al.* (1975) has explained that high genetic

variability is required in order to cope with the variety of environmental state to which they are subjected.

However, high level of genetic variability in local population may lead to population fragmentation among local populations in wide geographic range. In this case, it has to be careful to start restocking program by introducing individual from other populations. Fragmented population is suggested to have evolutionary adapted members that may be different among local populations. It is suggested that restocking may lead to the extinction of parental local populations.

Studies on the extent of individual variation have demonstrated that genotype has a significant effect on energy metabolism (Koehn, 1991). This implies that selection for good productions traits such as growth rate (important trait for selection of giant clams) should necessarily maintain some degree of variability. Mixing of genetically diverse broodstock of giant clams (*T. crocea* and *T. maxima*) would enhance genetic variation and assist the production of improved strain for mariculture. However, if the genetic variation leads to genetic differentiation among populations due to the result of evolutionary adaptation, mixing genetically different populations may result in the break up of co-adaptation gene complexes. This might result in the loss of the physiological capacities of the parental populations, as has been reported in the copepod *Tigriopus californicus* (Burton, 1986). With respect to restocking program, for the same reason, it may be more valuable to use broodstock from the same region or population to maintain the natural genetic resources. This study having shown high degree of genetic variation within local populations of both species of giant clams is likely providing a sufficient basis for local strain improvement.

Conclusion

The mitochondrial DNA cytochrome c oxidase I gene of *Tridacna crocea* and *T. maxima* showed high polymorphism and genetic variability within local populations. High levels of genetic variability are providing a sufficient basis for local strain improvement and restocking of both species.

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