HAYATI Journal of Biosciences, December 2007, p 162-166 Vol. 14, No. 4
ISSN: 1978-3019

SHORT COMMUNICATION

Molecular Phylogeny of Giant Clams Based on Mitochondrial DNA Cytochrome C Oxidase I Gene

AGUS NURYANTO[1‡*], DEDY DURYADI[1], DEDI SOEDHARMA[2], DIETMAR BLOHM[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, [2]Faculty of Fisheries and Marine Sciences, Bogor Agriculture University, Darmaga Campus, Bogor 16680, Indonesia
[3]Department of Biotechnology and Molecular Genetics, FB2-UFT, University of Bremen, Bremen, German

Received April 13, 2007/Accepted November 22, 2007

There is an uncertainty for the relationships among giant clam species of Tridacninae, in particular among species belongs to subgenus *Chametrachea* i.e. *Tridacna crocea*, *T. maxima,* and *T. squamosa* based on different genetic markers. This study examined the relationships among three species within subgenus *Chametrachea* compared to the previous studies. Neighbour Joining, Maximum Parsimony and Maximum Likelihood tree were constructed based on 455 bp of the mitochondrial DNA cytochrome c oxidase I gene from *T. crocea*, *T. squamosa*, *T. maxima*, *T. gigas,* and several sequences derived from Genbank for the outgroups. The results showed that giant clams formed a monophyletic group. Within *Tridacna* group, *T. crocea* was more closely related to *T. squamosa* than to *T. maxima* and they formed a monophyletic group. *T. crocea* and *T. squamosa* were sister taxa and sister group to *T. maxima* and *T. gigas*. Close affinity between *T. crocea* and *T. squamosa* was also supported by high similarity on nucleotide level (94.30%) and concordant with the results of the previous studies using mitochondrial 16S rRNA and nuclear 18S rRNA.

Key words: phylogenetic relationships, *Chametrachea*, cytochrome c oxidase I

The subfamily of Tridacninae is conspicuous bivalves inhabitanting coral reef across the Indo-Pacific regions (Lucas 1988). Total length of the adult individuals range from 15 cm (*Tridacna crocea*) up to 150 cm (*T. gigas*). There is an increasing interest to the clams, not only for their beautiful colouration but also tremendous decline on natural populations throughout their geographic range due to over-harvesting (Lucas 1988) and environmental deterioration (Pandolfi *et al*. 2003). Development of mariculture method and conservation technology of the clams has led a paramount research on clam biology (Copland & Lucas 1988). However, their systematic and phylogeny showed inconsistent results (Schneider & O'Foighil 1999). Resolved phylogeny is vital for marine resources conservation and management.

Eight extant species of giant clams are recognized. Two species belong to *Hippopus*: *Hippopus hippopus* Linnaeus 1759 and *H. porcellanus* Rosewater 1982 (Lucas *et al*. 1991). *Tridacna* is consisted of three subgenera i.e. (i) *Tridacna sensu stricto* with one species (*Tridacna gigas* Linnaeus 1758); (ii) *Chametrachea* with three species (*T. maxima* Röding 1798, *T. squamosa* Lamarck 1819 and *T. crocea* Lamarck 1819); and (iii) *Persikima* consists of *T. derasa* Röding 1798 and *T. trevoroa* Lucas, Ledua and Braley 1991. The present status of *T. rosewateri* Sirenko and Scarlato 1991 is still ambiguous. Benzie and Williams (1998) suggested that this species is a synonym of *T. squamosa*.

Tridacna (*Chametrachea*) is characterized by having wide byssal orifice and scales or corrugations on their shell. Adult individuals of *Chametrachea* are usually sessile and attached to hard surfaces throughout their lifespan (Lucas 1988). *Tridacna maxima* is often attached to coral rubbles. *Tridacna crocea* bores into coral head and only their mantle tissue is visible (personal observation). On the other hand, *T. squamosa* may attach to any hard surface and large individuals may lose their byssal attachment (Lucas *et al*. 1991).

Several studies were carried out on the phylogeny of giant clams. However, inconsistent results were produced about their relationships, especially within subgenus *Chametrachea*. For example, based on the partial sequences of 18S rDNA gene, Maruyama *et al*. (1998) obtained three phylogenetic trees i.e. (*T. maxima* (*T. crocea* + *T. squamosa*)), (*T. crocea* (*T. squamosa* + *T. maxima*)) and (*T. squamosa* (*T. crocea* + *T. maxima*). Each tree had a similar bootstrap value on the branching node of the *Chametrachea*. Based on partial sequences of mitochondrial 16S rDNA gene, Schneider and O'Foighil (1999) showed the same topology as the second tree of Maruyama *et al*. (1998). By using allozyme variations for 26 loci, Benzie and Williams (1998) confirmed that the major group currently recognized within Tridacninae based on morphological characters i.e. *Hippopus* and *Tridacna* and subgenera within *Tridacna*. Within the subgenus *Chametrachea* the relationship was (*T. squamosa* (*T. crocea* + *T. maxima*)).

‡Current address: Faculty of Biology, Jenderal Soedirman University, Jalan Dr. Suparno 63, Karangwangkal, Purwokerto 53123, Indonesia
*Corresponding author. Phone: +62-281-638794, Fax: +62-281-637100, E-mail: aghnia_unsoed@yahoo.com

Although the previous studies used different genetic markers, nevertheless, there is still a problem with the relationship of *T. crocea, T. maxima,* and *T. squamosa* that needs to be solved. Given that all giant clams are listed as vulnerable species (Wells 1997), therefore, it is imperative to understand their systematic and phylogeny so that appropriate decisions can be made concerning their conservation.

Here we studied the phylogeny of tridacnid clams using partial sequences of mtDNA cytochrome c oxidase I (COI) gene. This gene was chosen due to its high mutation rate (Hebert *et al*. 2003a), highly divergent among populations (Bucklin *et al*. 2003), and its broader phylogenetic sign (Hebert *et al*. 2003b). Therefore, it is suggested that COI gene fragment is suitable for studies the relationships among close related species and population genetic study. Clear discrimination among close related species was observed on various organisms (Bucklin *et al*. 1999; Bucklin *et al*. 2003; Klinbunga *et al*. 2005; Yosida *et al*. 2006). The aim of this study was to verify and provide better understanding of the relationships among giant clams species based on COI gene, especially for the species under *Chametrachea* subgenus.

Mantle tissues of seven species i.e. *T. crocea*, *T. maxima*, *T. squamosa*, *T. derasa, T. gigas, Hippopus hippopus,* and *H. porcellanus* were collected from several locations in the Indonesian Archipelago during the field trips in 2004 and 2005, i.e. from Padang, Pulau Seribu, Spermonde, Togian Islands, and Biak. Tissue samples of *T. squamosa* were also collected from the Red Sea in 2004 (Table 1). The determination of giant clams species were following Lucas (1988).

A small piece of mantel tissues was cut off from seven species carried out under water in order to minimise the sampling impact. Tissue samples were preserved in 96% of ethanol and stored at 4 °C. Total genomic DNA was isolated using Chelex® method following the protocols from Walsh *et al*. (1991). The extraction procedures were as follow: approximately 0.5 mg of chopped tissue was incubated in 100 µl 5% Chelex, 5 µl 100 mM dithiothreitol (DTT), and 4 µl of 9.85 or 10.3 mg/ml proteinase-K. The mixtures were then incubated at 54 °C and centrifuged at 1,000 rpm for minimum four hours. Afterwards, lysate was centrifuged at 13,000 rpm for three minutes to remove cell debris and the rest of Chelex granule. Subsequently, the supernatant was transferred into new tubes and incubated at 95 °C and centrifuged at 1,000 rpm for five minutes to inactivate the proteinase-K.

A fragment of COI gene was amplified using a pair of primers from Folmer *et al*. (1994) (forwards: LCO 1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and reverse: HCO 2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'). However, this primer could only amplify seven specimens of *T. crocea*. Hence, we designed new tridacnid specific primer (forwards: LCO: 5'-GGG TGA TAA TTC GAA CAG AA-3' and reverse: RCO: 5'-TAG TTA AAG CCC CAG CTA AA-3') based on *T. crocea* sequences obtained in a preliminary analysis. PCR reactions were carried out in a total volume of 50 µl contained approximately 10 pg 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 (*MolTaq*, Molzym GmBh & Co.KG, Germany). Thermal cycling was as follow: one cycle at 94 °C for 5 minutes, follows by 35 cycles of 1 minute at 94 °C, 1.5 minutes annealing temperature (at 50, 43, and 47 °C for *T. maxima* and *T. gigas*, *T. crocea,* and for *T. squamosa*, respectively) and one minute at 72 °C for extension. Final extension was carried out at 72 °C for five minutes.

Although we used tridacnid-specific primers, the COI gene could only be amplified for *T. crocea*, *T. maxima*, *T. squamosa,* and *T. gigas*. Those COI gene from remaining species of giant clams (*T. derasa, H. hippopus, H. porcellanus*) were tried to be amplified using new-designed universal COI primers (COIb-F: 5' ATC AYA WAG AYA TTG GHA S 3' and COIb-R: TGM CCA AAA AAY CAA AAYARR3'). These efforts were still unsuccessful eventhough the experiments were carried out in different annealing temperatures ranging from 40 °C up to 65 °C. Finally, we considered to continue our analysis only for *T. crocea*, *T. maxima*, *T. squamosa,* and *T. gigas*.

The PCR products were purified using the PeqGOLD cycle-pure kit (Peqlab Biotechnologie GmbH, Erlangen, 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

Table 1. List of species used in this study

Group	Species name	Sample number	Abbreviation	Location	Accession number	Collector
Ingroup	*Tridacna crocea*	Sample 1	TcPS8301	Pulau Seribu	EU003608	Nuryanto A
		Sample 2	TcSp0301	Spermonde	EU003606	
		Sample 3	TcSp0302	Spermonde	EU003607	
		Sample 4	TcBk8804	Biak	EU003609	
	Tridacna maxima	Sample 1	TmPa4601	Padang	EU003610	
		Sample 2	TmPS8301	Pulau Seribu	EU003614	
		Sample 3	TmSp0301	Spermonde	EU003611	
		Sample 4	TmTI2901	Togian Islands	EU003612	
		Sample 5	TmBk9006	Biak	EU003613	
	Tridacna gigas	Sample 1	T gigas	Togian Islands	EU003616	
	Tridacna squamosa	Sample 1	TsRS9501	Red Sea	EU003615	Roa-Quiaoit HAF
	Tridacna crocea			Taiwan	DQ269479	Tang TC
Outgroup	*Parvicardium*	-	-	European	AF120664	Giribet G
	Ruditapes	-	-	Korea	AY874536	Kim JJ *et al.*
	Mytilus	-	-	-	AY484747	Hoffmann *et al.*