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Original Article

Study on genetic variability of blood ark, *Anadara cornea* at east coast of peninsular Malaysia using random amplified polymorphic DNA: A preliminary assessment

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Abstract

The genetic variability among individuals of *Anadara cornea* from Setiu Wetlands, Terengganu was examined by using random amplified polymorphic DNA (RAPD) technique. The genomic DNA was extracted from the clam by using Macherey-Nigel DNA kit and was amplified by RAPD technique with five primers (OPA O3, OPA O9, OPA 17, OPA 19 and OPA 20) for twenty samples of *A. cornea*. A total of 79 RAPD bands with 78 polymorphic bands (98%) with size ranging from 150-3700 bp were identified from the population indicate that the population of *A. cornea* has high level of polymorphism due to low inbreeding factor within a population. High polymorphism of *A. cornea* revealed that this species has genetically variable. The results of this study can be useful to the sustainable management of wild stocks of this species. Further study should be done for a better understanding about variation and also for conservation and management of this species.

Keywords: *Anadara* sp, Blood ark, Clam, Genetic variability, RAPD, Setiu Wetland

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Introduction

Bivalve is a major class of molluscs and can be found in fresh water and salt water. Bivalve body enclosed in a two-lobed mantle, possess a shell divided into two halves, hinged at the mid dorsal line and have two adductor muscle to protect the animal (Hick man and Kats, 2004). *Anadara cornea* is one of the bivalves in Phylum Mollusca in the family Arcidae, which consists of about 200 species (Zupan et al., 2012; Mohd and Bachok, 2017). *Anadara* spp, is one of the ark shells or 'blood cockle' has significant economic value in fisheries (Power et al., 2004; Tanaka and Aranishi, 2013). *A. cornea* inhabit in mud and sand and distributed from Thailand to the Philippines; north to Japan and south to Indonesia and can be found in Turkey, India and China (Poutiers, 1998; Erdogan et al., 2010; Prabhakaran et al., 2012; Yennawar and Tudu, 2014). *A. cornea* in Setiu Wetlands are harvested without any control from the authorities and the wetlands can be accessed by anyone. Unregulated and uncontrolled harvesting, in long term, will lead to over-exploiting and threatening the resilience of the bivalve populations (Rodrigues, 2013; Dolorosa and Dangan-Galon, 2014, Ibrahim et al., 2018). Over exploitation can cause the decrease of genetic diversity of a population and result the reduction of the survivability of a species (Trisyani and Budiman,

2015). Assessment of genetic variation is an important step towards the implementation of species conservation strategies (Ward, 2006). Genetic diversity and population structure are critical factors for long-term survival of a species and play important role in the conservation and management of wild species.

A number of applicable molecular tools for the detection of genetic variation within species have been developed and Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) is one of the molecular genetic markers and it was widely used for studying the genetic variability over the world This techniques was chosen as it is rapid and simple and allow the examination of genomic variation without prior knowledge of the genome and it's require only small quantity of DNA for determining the genetic variability in many organisms (Williams et al., 1990; Hadrys et al., 1992). In Malaysia, studied about genetic variability and species-diagnostic of oyster (*Crassostrea iredalei)* was conducted using RAPD in Peninsular Malaysia (Wan Bayani, 2003). RAPD was also used to study the genetic variation due to heavy metals contamination in *Perna veridis* collected from Johor (Yap et al., 2007). RAPD technique was also used to investigate the variation of *A. granosa* in west coast of Peninsular Malaysia (Chee et al., 2011). This technique was also used to detect genetic diversity and species-diagnostic markers in five species of oysters in Thailand (Klinbunga et al., 2001). RAPD markers have been applied to study genetic variability related with heavy metals contamination of bivalve, *Coelatura* spp in Egypt (El Assal et al., 2014).

In Malaysia, studied on genetic variability of *A. cornea* had been done (Wan Omar and Kassim, 2015) but only used six individuals of samples and two primers. This is not enough to explain the variability of the species. Therefore, this study was conducted to assess genetic variability of *A. cornea* using 20 individuals and five primers in this wetland by using RAPD-PCR technique.

Material and Methods

Sample collection

The samples of *A. cornea* were collected from the Setiu Wetland, Setiu, Terengganu. Twenty individuals were used in this study.

DNA extraction and RAPD

DNA was extracted from muscle tissue based on the procedure of the Macherey-Nigel DNA kit. The quantity of DNA was measured by using BioDrop™ µLITE dsDNA. 20 RAPD primers from 1st Base (with 60% - 70% G-C) content was screened. Only the primers that have clarity of the profile, sharpness and the existence of polymorphism were chosen for further study (D'Amato and Corach, 1997). Total reaction volume of 25µl was used with the final concentration containing 1x reaction buffer, 50 ng genomic DNA, 1 mM of magnesium chloride, 1mM of dNTPs and 2.5 mM primers and 2.5 Units of Taq DNA polymerase. The amplification was programmed at 45 cycles for 30s denaturation at 94°C, 30s of annealing temperature at 36°C, 1 min of primers extension at 72°C and final extension of 2 min at 72°C. PCR product was electrophoresed on 1.5% (w/v) agarose gel in 1x TBE buffer at 70 V for 1 and 30 minutes. The gel was stained with SYBR Safe and photographed with Gel Doc™XR.

Data analysis

The numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) Version 2.1 was used in this study. Molecular weight of the bands were estimated based on the standard DNA banding pattern from 1 Kb ladder plus (Invitrogen). Clear bands were scored as present (1) or absent (0) at particular position or distance migrated on the gel. These bands were considered as polymorphic when they were absent in some sample in frequency greater than 1% (Jorde, 1995). For scoring the banding pattern, the presence of band was scored as number '1' and absence was scored as '0'. The index of similarity between individuals was calculated. The formula being used is: SI = 2 Nxy/ (Nx + Ny). Nxy is the number of fragments shared by individual x and y. For Nx and Ny, both are the total number of bands scored in x and y respectively. So, the similarity was calculated based on method proposed by Nei and Li (1979). For genetic distance, the index similarity was used to calculate the values of genetic distance and to construct the dendrogram. The dendrogram as visual representation that is used to provide the relationship of different individuals of *A. cornea*. The dendrogram from NTSYS-pc was constructed by Unweighed Pair-Group Method of Arithmetic (UPGMA) which is employing the Sequential, Agglomerative, Hierarchical and Nest Clustering (SAHN) (Rohlf, 1994).

Results and Discussion

In this study, five primers (OPA 03, OPA 09, OPA 17, OPA 19 and OPA 20) were used and applied on 20 individuals of *A. cornea* for DNA amplification. The results showed the different primers generated different number of fragments and length products of DNA amplification as shown in Table 1.

Table-1: Total number of fragments, number of polymorphic fragments, percentage of polymorphic and length of fragments, of *A. cornea* generated from OPA 03, OPA 09, OPA 17, OPA 19 and OPA 20 for Setiu Wetlands.

Primer	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphic (%)	Length of fragments
OPA 03	17	17	100	250-1500
OPA 09	19	19	100	250-1500
OPA 17	7	6	86	500-2000
OPA 19	17	17	100	150-1800
OPA 20	19	19	100	300-3700
Total	79	78	Average = 98	

In this study the similarity index for *A. cornea* from Setiu Wetland was ranged from 0.16 to 0.67. The low similarity index indicated high variability between individuals in that area. The analysis of UPGMA cluster of *A. cornea* based on the genetic distance level generated from Nei and Li's indices as shown in Figure 2. Genetic distance levels of *A. cornea* from Setiu Wetlands ranged from 0.33 to 0.84.

At the genetic distance 0.67 there were two main clusters of *A. cornea*. The first cluster consisted of the individuals 1, 2, 4, 6, 7, 8, 10, 3 and 5. The second cluster 9 and 13. Meanwhile at the genetic distance 0.64 there were another two main clusters. The first consisted of the individuals 11, 17, 19, 12, 14, 18, 15 and 16. The second cluster consisted only one individual, it was 20.

The percentage of polymorphism of *A. cornea* at Setiu Wetlands was 98% higher than previous studied in Setiu Wetlands by Wan Omar and Kassim (2015) showed that the percentage of polymorphism of *A. cornea* was 78%. A high polymorphism of *A. cornea* in Setiu Wetlands showed that this species in such locality are highly heterogeneous due to low level inbreeding. As the comparison, in another RAPD study for some bivalve such as two oyster species (*Crassostrea iredalei* and *C. belcheri*) in Thailand showed that high percentage of polymorphism with 53% and 77%, respectively (Klinbunga, 2001).

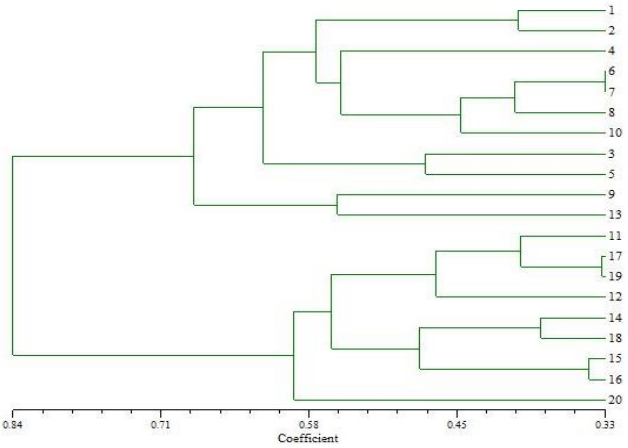

Figure-2: UPGMA cluster analysis based on genetic distance generated from Nei and Li's indices *A. cornea* from Setiu Wetlands. Data of RAPD generated by primers OPA 03, OPA 09, OPA 17, OPA 19 and OPA 20. Individuals (1-20).

Wan Bayani (2003) studied the polymorphism of *C. iredalei* in east coast of Peninsular Malaysia also found a high level of polymorphism which ranged from 71% to 84%. Joaqim et al. (2009) reported that high polymorphism of bivalve, *Venerupis senegalensis* in two populations of Portugal (Ria Farmosa, 86.5%; Ria de Aveiro, 87%). The population of *A. cornea* in Setiu Wetlands was showed high level polymorphism of individuals within its population due to low level of inbreeding. The high genetic variability suggested that the population of this species had a gene pool with sufficient genetic plasticity to support changes in the environmental changes (Joaqim et al., 2009). High level of genetic variability in invertebrates strongly depend on its life form, geographic range and larval dispersal mechanism (Ward, 2006). It also supported by Nie et al. (2015) stated that a high level of genetic variation is essential for long-term survival of populations and can determines their ability to adapt in changing environments.

Conclusion

We found that the high levels of polymorphism in *Anadara cornea* at Setiu Wetlands by using RAPD marker. The high polymorphism revealed that the individuals of *A. cornea* were genetically variable due to low level of inbreeding. This result provide valuable

genetic information towards its conservation and management of this species.

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Contribution of Authors

Omar WBW: Data collection and manuscript writing
Mohamad, F: Designed research methodology
Ibrahim, NE: Data collection and analysis

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