Malays. Appl. Biol. (2019) 48(1): 15–19

STUDY ON GENETIC VARIABILITY OF MUD CRAB, *Scylla olivacea* AT EAST COAST OF PENINSULAR MALAYSIA USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD): A PRELIMINARY ASSESSMENT

WAN BAYANI WAN OMAR[1*], FARIDAH MOHAMAD[1], IKHWANUDDIN @ POLITY ABDULLAH[2] and MOHD FIRDAUS ZAINAL ABIDIN[1]

[1]*School of Marine and Environmental Sciences, 21030, Kuala Terengganu, Terengganu*
[2]*Institute of Tropical Aquaculture, 21030, Kuala Terengganu, Terengganu*
[*]*E-mail: bayani @umt.edu.my*

Accepted 10 January 2019, Published online 20 March 2019

ABSTRACT

Scylla olivacea is one of the mud crabs species and become source of economic income especially for local fishermen in Setiu Wetlands. Nevertheless, the genetic variability of this species are still poorly studied and need to be further clarified. Thus, the Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique was used to examine the genetic variability among individuals of *S. olivacea* from Setiu Wetlands, Terengganu. The genomic DNA was extracted from ten samples of mud crab by using DNeasy kit. Twenty reproducible RAPD fragments generated by OPA 08 and OPA 10 with 17 fragments were polymorphic. The percentage of polymorphic bands was 85% indicates that the population of *S. olivacea* has high level of polymorphism due to low inbreeding factor within a population. High polymorphism of *S. olivacea* revealed that this species are genetically variable. Further study should be done for a better understanding about variation and also for conservation and management of this species.

Key words: Genetic variability, Mud crab, RAPD, *Scylla olivacea*, Setiu Wetlands

INTRODUCTION

Mud crabs from the genus *Scylla* and family Portunidae, have important economic values and is one of the edible food species (Ramalingam *et al*., 2015). These species can be found in the Indo-Pacific regions usually found in sheltered waters particularly estuaries, tidal flats and mangrove areas (Fuseya & Watanabe, 1996; Jirapunpipat *et al*., 2009). These species are large and tasty making them a highly scarce supply and also subject to overexploitation due to high demand (Le Vay, 2001) and resulted in decreasing in both landing and maximum size of capture (Kosuge, 2001).

Only three mud crab species are found in Setiu Wetlands which are; *Scylla olivacea*, *S. paramamosain* and *S. tranquebarica* (Ikhwanuddin *et al*., 2014a). Nowadays, crab industry has become an import aquaculture field after shrimp and fish productions (Ramalingam *et al*., 2015). Conservation of species with important economic value has become important all over the world. Population of these species is affected by a critical loss of mangrove forest because of deforestation and urban development (Spalding *et al*., 1997). To date, in Setiu Wetlands, studies conducted on *S. olivacea* was mainly focused on species diversity, carapace width-body weight relationship, size distribution and sex ratio, size of maturity and effect of water salinity on mating success (Ikhwanuddin *et al*., 2010a; Ikhwanuddin *et al*., 2010b; Ikhwanuddin *et al*., 2014b). There is lack of information regarding the genetic variability of this species, especially in Setiu Wetlands.

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 (Williams *et al*., 1990). This techniques was chosen as it is rapid and simple and have benefits with no prior knowledge of the genome and it requires only small quantity of DNA for determining

* To whom correspondence should be addressed.

the genetic variability in many organisms especially mud crabs (Hadrys *et al*., 1992; Klinbunga *et al*., 2000; Ramalingam *et al*., 2015). RAPD also has been used in the identification of the correct broodstock species for successful culture of any *Scylla* sub species in Chennai, India (Ramalingam *et al*., 2015) and another study was conducted using RAPD marker by Klinbunga *et al*. (2000) about the genetic diversity of *Scylla* in Eastern Thailand. In Malaysia, studied has been done about genetic variability of mud crabs (*S. olivacea*) by using mitochondria DNA (Cytochrome c oxidase I gene) in which they compared the population structure of this species for six localities in Peninsular Malaysia and one of the localities was Terengganu but did not mention where the samples came from (Hurul Adila-Aida *et al*., 2013). Therefore, this study was conducted to assess the genetic variability of *S. olivacea* in Setiu Wetlands by using RAPD-PCR technique.

MATERIALS AND METHODS

Sample collection

The samples of *S. olivacea* were collected from the Setiu Wetlands, Terengganu. A total of ten individuals were used in this study and labelled as CP1 to CP10, respectively.

DNA extraction and PCR amplification

DNA was extracted from muscle tissue based on the procedure of the DNeasy Blood and Tissue Kit (Qiagen's) protocol. The quantity of DNA was measured by using BioDrop™ μLITE dsDNA. Two RAPD primers, OPA 08 (5'- GTGACGTAGG -3') and OPA 10 (5'- GTGATCGCAG -3') from 1st Base were used. Only the primers that have clarity of the profile were chosen for further study (D'Amato and Corach, 1997). The amplification was carried out in a 25 μl reaction volume containing 1x reaction buffer, 50 ng genomic DNA, 1 mM of $MgCl_2$, 1mM of dNTPs and 2.5 mM primers and 2.5 Units of *Taq DNA polymerase*. The amplification was programmed at 35 cycles of denaturation at 94°C for 15s, annealing at 36°C for 30s, extension at 72°C for 1 min and a final extension at 72°C for 2 mins. PCR product was electrophoresed on 2% (w/v) agarose gel in 1x TBE buffer at 70 V for 1 hour and 30 min. The gel was stained with SYBR Safe and photographed with Gel Doc™XR.

Data analysis

The Molecular weight of the bands were estimated based on the standard DNA banding pattern from 1 Kb ladder plus (Invitrogen) and recorded in a binary matrix (0/1) to represent as present (1) or absent (0) of a particular band for each individual. These bands were considered as polymorphic when they were absent in some sample in frequency greater than 1% (Jorde, 1995). The index of similarity between individuals was calculated. The formula being used is: SI = 2 Nxy/(Nx + Ny). Nxy is the number of fragment shared by individual x and y. For Nx and Ny, both are the total number of band scored in x and y respectively. So, the similarity was calculated based on the method proposed by Nei & Li (1979). For genetic distance, the index similarity was used to calculate the values of genetic distance and to construct the dendrogram. The dendrogram is used as a visual representation to provide the relationship of different individuals of *S. olivacea*. 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

A survey on the amount of mud crab species for sale at various stalls in Setiu Wetlands found that *S. olivacea* is the most abundant of mud crab species. Therefore, *S. olivacea* had been chosen for their genetic variability study. Two primers (OPA 08 and OPA 10) were used for 10 individuals of *S. olivacea* for DNA amplification. Results showed that different primers generated the different number of fragments and the length products of DNA amplification is shown in Table 1. There were 20 fragments generated by both primers. OPA 08 generated 12 fragments (Figure 1) and OPA 10 generated 8 fragments (Figure 2). The size of bands ranged from

Table 1. Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of *S. olivacea* generated from OPA 08 and OPA 10

Primer	Number of fragments	Size of fragments (bp)	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphic (%)
OPA 08	2–13	200–5000	12	11	91
OPA 10	5–15	250–3300	8	6	75
Total	–	200–5000	20	17	85

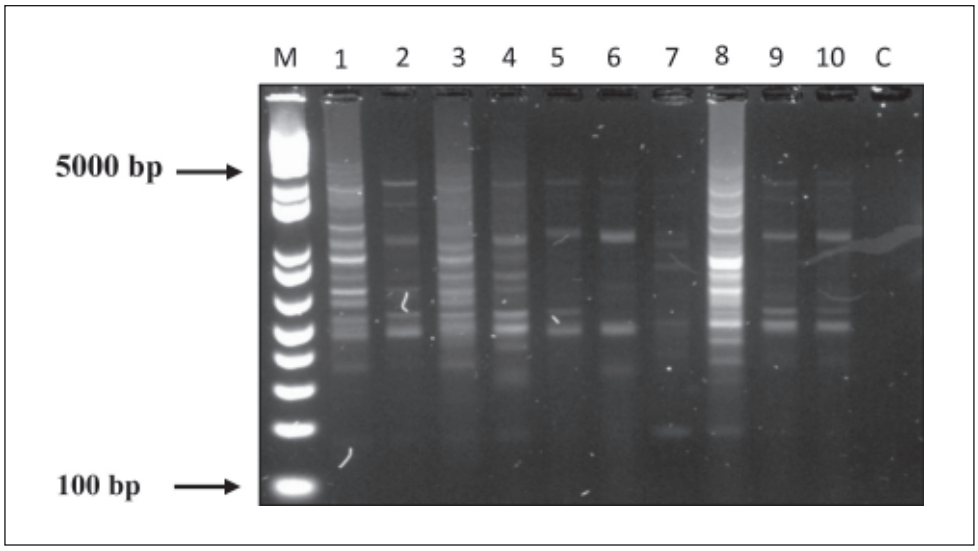

Fig. 1. Banding patterns of RAPD fragments of *S. olivacea* using primer OPA 08 (Lane M is maker 1Kb plus DNA ladder. C is control. Individuals CP1 to CP10 is represented by number 1 to 10, respectively.

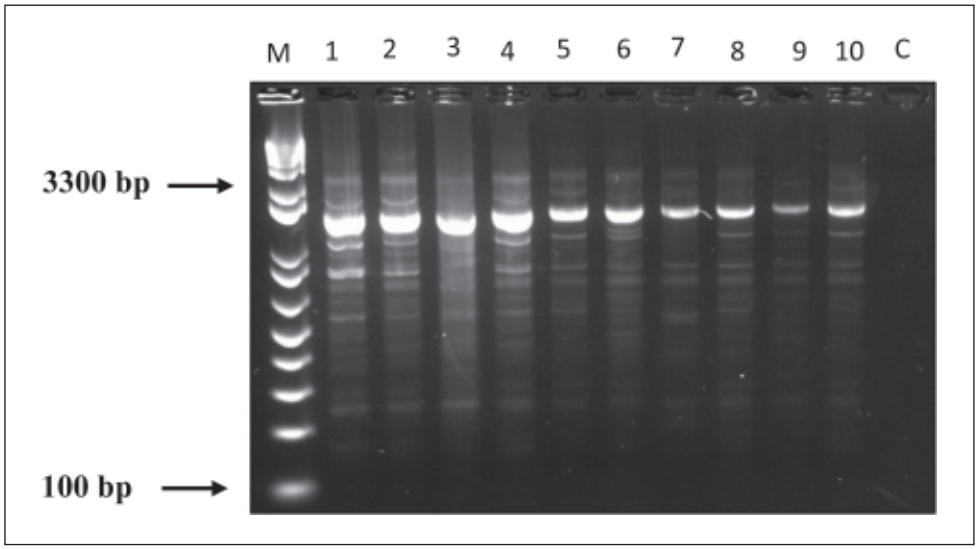

Fig. 2. Banding patterns of RAPD fragments of *S.olivacea* using primer OPA 10 (Lane M is marker 1Kb plus DNA ladder. C is control. Individuals CP1 to CP10, is represented by number 1 to 10, respectively.

200–5000 bp. The existence of polymorphic markers indicated diversity among the individuals at genomic level (Williams *et al*., 1990).

In this study the similarity index for *S. olivacea* was ranged from 0.35 to 0.90 as shown in Table 2. Analysis of UPGMA cluster of *S. olivacea* based on the genetic distance level generated from Nei and Li's indices as shown in Figure 3. Genetic distance levels of *S. olivacea* from Setiu Wetlands ranged from 0.1 to 0.65 as compared to Thailand was ranged from 0.17 to 0.19 (Klinbunga *et al*., 2000) which was much lower than in Setiu Wetlands.

The percentage of polymorphism for *S. olivacea* at Setiu Wetlands was 85% as high as that previously reported in another studies in Thailand which average percentage of polymorphic bands of three species of mud crabs (*S. serrata*, *S. oceanica*, *S. tranqueibarica*) between two population in Thailand was relatively high and comparable for each population (64.81%–77.59%) (Klinbunga *et al*., 2000) but much lower in India was 31%–37%, *S. tranqueibarica* (Ramalingam *et al*., 2015). As comparison, RAPD study in other crustacean indicated the following results; 22.3%–40.9% in shrimp, *Metapenaeus dobsoni* (Mishra *et al*., 2009), 20%–23.3% in *Penaeus chinensis* (Zhang *et al*., 2001) and 51.1% to 57.7% in tiger shrimp, *Penaeus monodon*. The high level of polymorphism in *S.*

Table 2. The similarity index of *S. olivacea* generated from OPA 08 and OPA 10

Sample	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9	CP10
CP1										
CP2	0.7500									
CP3	0.5500	0.5000								
CP4	0.6500	0.8000	0.6000							
CP5	0.5000	0.7500	0.4500	0.6500						
CP6	0.5000	0.7500	0.4500	0.6500	0.9000					
CP7	0.4500	0.5000	0.6000	0.5000	0.7500	0.7500				
CP8	0.6000	0.3500	0.5500	0.4500	0.4000	0.4000	0.5500			
CP9	0.4000	0.5500	0.5500	0.4500	0.8000	0.7000	0.6500	0.4000		
CP10	0.5000	0.6500	0.4500	0.5500	0.9000	0.8000	0.6500	0.5000	0.9000	

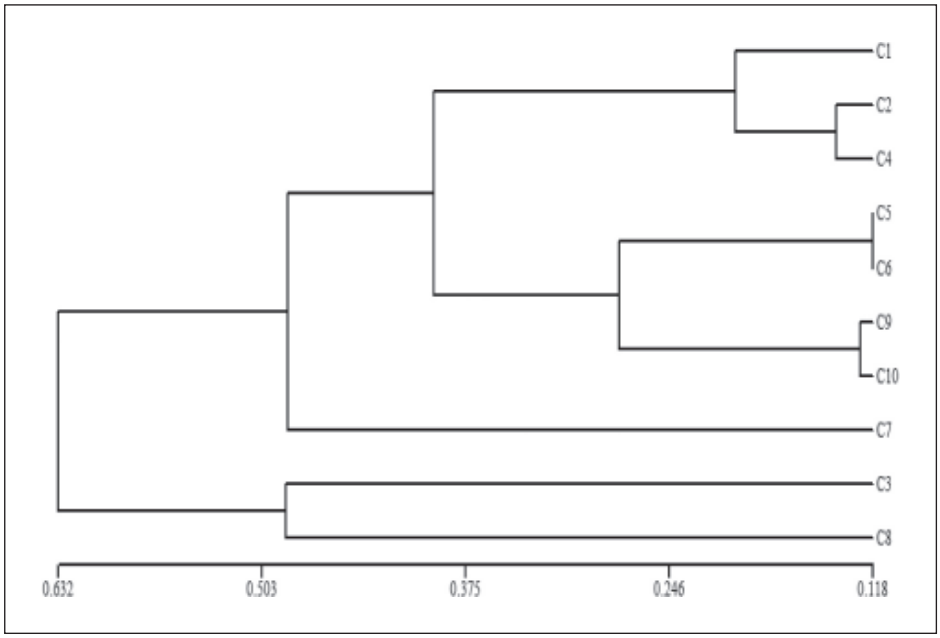

Fig. 3. UPGMA cluster analysis based on genetic distance generated from Nei and Li's indices *S. olivacea* from Setiu Wetlands. Data of RAPD generated by primers OPA 08 and OPA 10. Individuals C1-C10).

olivacea population indicates that inbreeding may not occur or occurred at small rate, and also showed that this species is highly heterogeneous which postulated that the cross breeding among diverse individual had occurred in the population (Wan Bayani, 2003). It was supported by Parenrengi (2001) stated that the population which have high polymorphic fragment indicates the level of breeding is low among individuals of each population.

CONCLUSION

Our results point out that the RAPD markers successfully amplified the DNA of *S. olivacea* at Setiu Wetlands. The high polymorphism revealed that the individuals of *S. olivacea* are genetically variable. The findings of this study should contribute to better understanding on genetic variability which would lead to sustainable management and conservation of this species for broodstock selection and selective breeding programs in the future.

ACKNOWLEDGEMENTS

We thank the School of Marine and Environmental Sciences and Central Laboratory of University Malaysia Terengganu for providing necessary facilities for the completion of this project.

REFERENCES

D'amato, M.E. & Corach, D. 1997. Population genetic structure in the fresh water anomuran *Aegla jujuyana* by RAPD analysis, *Journal of Crustacean Biology*, **17**: 269-274.

Fuseya, R. & Watanabe, S. 1996. Genetic variability in the mud crab genus *Scylla* (Brachyura: Portunidae). *Fish Science*, **62(5)**: 705-709.

Hadrys, H., Balick, M. & Shierwater, B. 1992. Application of Random Amplified Polymorphic DNA (RAPD) in Molecular Ecology. *Molecular ecology*, **1**: 55-63.

Hurul Adila-Aida, M.R., Siti Azizah, M.N., Khairun, Y. & Darlina, M.N. 2013. Mitochondrial DNA diversity of mud crab *Scylla olivacea* (Portunidae) in Peninsular Malaysia: a preliminary assessment. *Molecular Biology Report*, **40**: 6407-6418.

Ikhwanuddin, M., Bachok, Z., Hilmi, M.G. & Azmie, G. 2010a. Species diversity, carapace width-body weight relationship, size distribution and sex ratio of mud crab, genus *Scylla* from Setiu Wetlands of Terengganu coastal waters, Malaysia. *Journal of Sustainability Science and Management*, **5**: 97-109.

Ikhwanuddin, M., Bachok, Z., Faizal, M. & Azmie, G. 2010b. Size of maturity of mud crab *Scylla olivacea* (Herbst, 1796) from mangrove areas of Terengganu coastal waters. *Journal of Sustainability Science and Management*, **5**: 134-147.

Ikhwanuddin, M., Munafi, A. & Ambak, M.A. 2014a. Mud Crabs of Setiu Wetlands. Kuala Terengganu. 146pp.

Ikhwanuddin, M., Baiduri, S.N., Norfaizza, W.W. & Abol-Munafi, A.B. 2014b. Effect of water salinity on mating success of orange mud crab, *Scylla olivacea* (Herbst, 1796) in captivity. *Journal of Fisheries and Aquatic Science*, **9**: 134.

Jirapunpipat, K., Yokota, M. & Watanabe, S. 2009. The benefit of species-based management of sympatric mud crabs migrating to a common fishing ground. ICES *Journal of Marine Science*, **66**: 470-477.

Jorde, L.B. 1995. Populations specific genetic markers and diseases. In Biology and Biotechnology: A comprehensive desk reference, ed. R.A. Meyers, 724-728. New York: VCH Publisber, Inc.

Klinbunga, S., Boonyapakdee, A. & Pratoomchat, B. 2000. Genetic Diversity and Species Diagnostic Markers of Mud Crabs (Genus *Scylla*) in Eastern Thailand Determined by RAPD Analysis. *Marine Biotechnology*, **2(2)**: 180-187.

Kosuge, T. 2001. Brief assessment of stock of mud crab *Scylla* spp. and proposal for resources management. Seikai National Research Institute, **35(2)**: 145-148.

Le Vay, L. 2001. Ecology and management of mud crabs *Scylla* spp. *Asian Fisheries Science*, **14**: 101-11.

Mishra, P.S., Chaudari, A., Krishna, G., Kumar, D. & Lakra, W.S. 2009. Genetic diversity in *Metapenaeus dobsoni*. *Biochemical Genetics*, **47**: 421-426

Nei, M. & Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proceeding of National Academy of Sciences USA*, **7**: 5269-5273.

Parenrengi, A. 2001. Study on genetic variability of Groupers (*Epinephelis* spp.) from Malaysia and Indonesia waters using PCR-RAPD Analysis. Faculty of Science and Technology, Universiti Malaysia Putra.

Ramalingam, K., Bharathi, R.U.D. & Sri, K.N. 2015. Sample Population Genomic Study of RAPD-PCR DNA Analysis in *Scylla serrata* and *Scylla tranquebarica* Collected from Two Niches of Chennai Coast. *International Journal of Recent Scientific Research,* **6(5)**: 183-190.

Rohlf, F.J. 1994. NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.1. Department of Ecology and Evolution. Exeter Software, New York. 11733- 2870 pp.

Tassanakajon, A., Pongsomboon, S., Rimphanitchayakit, V., Jarayabhand, P. & Boonsaeng, V. 1998. Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. *Journal of Marine Biotechnology*, **6**: 249-254.

Spalding, M., Blasco, F. & Field, C. 1997. World Mangrove Atlas. *The International Society Mangrove Ecosystems*. Okinawa. 198 pp.

Wan Bayani, W.O. 2003. Study on genetic variability of oyster (*Crassostrea iredalei*, Faustino) in Peninsular Malaysia using RAPD-PCR technique, Faculty of Agrotechnology and Food Science. Universiti Malaysia Terengganu.

William, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nuclei Acid Research*, **18**: 6531-6535.

Zhang, Z., Shi, T., Kong, J., Liu, P., Liu, Z., Meng, X. & Deng, J. 2001. Genetic diversity in Penaeus chinensis shrimp as revealed by RAPD technique. *Progress in Natural Science*, **11(6)**: 332-338.