Malays. Appl. Biol. (2017) 46(2): 145–148

RESEARCH NOTE

HIGH GENETIC SIMILARITIES BETWEEN *Portunus pelagicus* FROM TELUK KEMANG, PORT DICKSON AND BATU PAHAT, JOHOR AS INFERRED FROM MITOCHONDRIAL *Cytochrome c Oxidase* I (COI) SEQUENCES

INTAN ROHAYU SUKIMIN[1], YUZINE B. ESA[1,2*] and S. M NURUL AMIN[1]

[1]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Institute of Biosciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[*]*Email: yuzine@upm.edu.my*

Accepted 8 May 2017, Published online 27 June 2017

The blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) is an edible crab that occurs in shallow tropical and temperate coastal and estuarine waters throughout the Indo-West Pacific region, from east Africa to Japan and northern New Zealand (Kailola *et al.,* 1993; Romano & Zeng, 2006). Within Peninsular Malaysia, this species is distributed along the coastal lines of both the Strait of Malacca (west) and the South China Sea (east) (Naiyanetr, 1998). The *P. pelagicus* supports the commercial fisheries and its important component to recreational fisheries in Malaysia and other countries (Sumpton *et al*., 1989). This species attains high demand in the internal and export market due to their delicacy (Maheswarudu *et al.,* 2008). An occurrence of an increasingly large proportion of small sizes of wild-caught Portunid crabs at present suggests overexploitation of this species.

The use of molecular marker such as mitochondrial DNA (mtDNA) had been one of the successful methods for determining the genetic diversity of population and species (Esa *et al*., 2008; Kamarudin & Esa, 2009; Ponzoni *et al.,* 2010). Mitochondrial DNA marker was also more useful and informative compared to nuclear DNA in term of sequence divergence because of its rapid evolution, lack of introns, less exposure to recombination and high copy number (Luo *et al.,* 2011). Currently, no study has been conducted on the genetic diversity and differentiation of *P. pelagicus* in Peninsular Malaysia. Hence, this study was conducted to determine the level of genetic differences between two *P. pelagicus* populations from Teluk Kemang, Port Dickson and Batu Pahat, Johor.

Samples of *P. pelagicus* were collected from Teluk Kemang, Port Dickson (PD) and Batu Pahat, Johor (BP) between September 2015 and February 2016. A total of 63 samples (41 samples from Batu Pahat and 22 samples from Teluk Kemang) were collected. Muscle tissue was taken from the cheliped manus of each sample and preserved in a collecting tube containing 95% ethanol. The remaining crab samples were stored in -20ºC until used for further analysis. Genomic DNA was extracted from each sample using the Promega Genomic DNA Purification Kit Protocol (Promega) following the manufacture's protocol.

Approximately 650 bp fragment of the mitochondrial COI gene was amplified via PCR with the universal primers COIa (5'-AGTATAAGCGTCT GGGTAGTC-3') and COIf (5'-CCTGCAGGAGG AGGAGATCC-3') acquired from Kessing *et al.* (1989). A total of 2 µl of DNA template was amplified in a reaction mixture containing 5 µl of 5X buffer (50 mM), 3 µl of $MgCl_2$ (2mM), 0.5 µl of dNTPs (8 mM) (Promega), 0.5 µl of each primer and 0.2 µl of *Taq* DNA polymerase (Promega). The reaction mixture was then adjusted to a final volume of 25 µl with ddH_2O. The thermal cycle was set as follows: initial denaturation at 94ºC for 2 min; 30 cycles of denaturation at 94ºC for 1 min; annealing at 46ºC for 1 min; extension at 72ºC for 1.5 min; and a final extension at 72ºC for 2 min.

* To whom correspondence should be addressed.

PCR products were electrophoresed on 1% agarose gel stained with GelRed, performed at 75V for 30 min and photographed under UV light. Bench Top 100 bp DNA ladder (Promega) was used as a standard DNA size marker (Promega). The PCR products were further purified using Wizard® SV Gel and PCR Clean-Up System (Promega). All the purified PCR products were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

The Chromas 2.4.4 (Technelysium) software was used to view the sequences and chromatograms. The multiple alignments of the sequences were subsequently conducted using the Clustal X program version 2.0.10 (Larkin *et al.*, 2007). The pairwise genetic distance between haplotypes was calculated using the Kimura two-parameter evolution model (Kimura, 1980) implemented in MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic confidence was estimated by bootstrapping with 1000 replicate datasets (Felsenstein, 1985). Phylogenetic relationships were inferred by constructing a Neighbor-joining (NJ) tree (Saitou & Nei, 1987) method with 1000 randomizations. Two sequences of crab species (*Callinectus sapidus* and *Charybdis feriatus* (accession numbers KC789112.1 and EU284140.1, respectively) were used as outgroups.

A total of 650 bp of the mitochondrial COI gene were obtained from 63 individuals of *P. pelagicus* from the two populations and after alignment and removal of gaps, a final sequence length of 599 bp were obtained of which only 12 (2.0%) variable sites, including 10 (1.7%) parsimony-informative characters were identified. A total of six and five haplotypes (NHap) were found in Teluk Kemang and Batu Pahat, respectively (Table 2). The pairwise genetic distance values between haplotypes were low, ranging from 0.1% to 1.0% (Table 1) as well as low nucleotide diversity values in both populations (0.08%; Table 2). A total of 43 individuals shared a single common haplotype (haplotype A) with the highest frequency of 68.25% (Table 2). A genetic study on *Clarias macrocephalus* populations showed that shared haplotypes of more than 50.0% frequency suggested that the species has a continuous gene flow and probably shared a common origin (Nazia *et al.,* 2010). Phylogenetic analysis showed all haplotypes sequences were clustered and mixed together in the phylogenetic tree without any obvious clustering between populations (Figure 1).

Table 1. Pairwise genetic distance among six haplotypes of *P. pelagicus*

	1	2	3	4	5	6
1. Haplotype A	–					
2. Haplotype B	0.005					
3. Haplotype C	0.010	0.005				
4. Haplotype D	0.008	0.007	0.002			
5. Haplotype E	0.019	0.014	0.008	0.010		
6. Haplotype F	0.017	0.015	0.010	0.008	0.002	–

Table 2. Distribution of haplotype frequencies in the two *P. pelagicus* populations

Haplotype	Port Dickson (PD)	Batu Pahat (BP)
Haplotype A	0.45 (10)	0.80 (33)
Haplotype B	0.09 (2)	–
Haplotype C	0.04 (1)	0.07 (3)
Haplotype D	0.03 (1)	0.03 (1)
Haplotype E	0.27 (6)	0.07 (3)
Haplotype F	0.09 (2)	0.03 (1)
Nucleotide diversity	0.010	0.011
No. of haplotype	6	5
Haplotype diversity	1.000	1.000

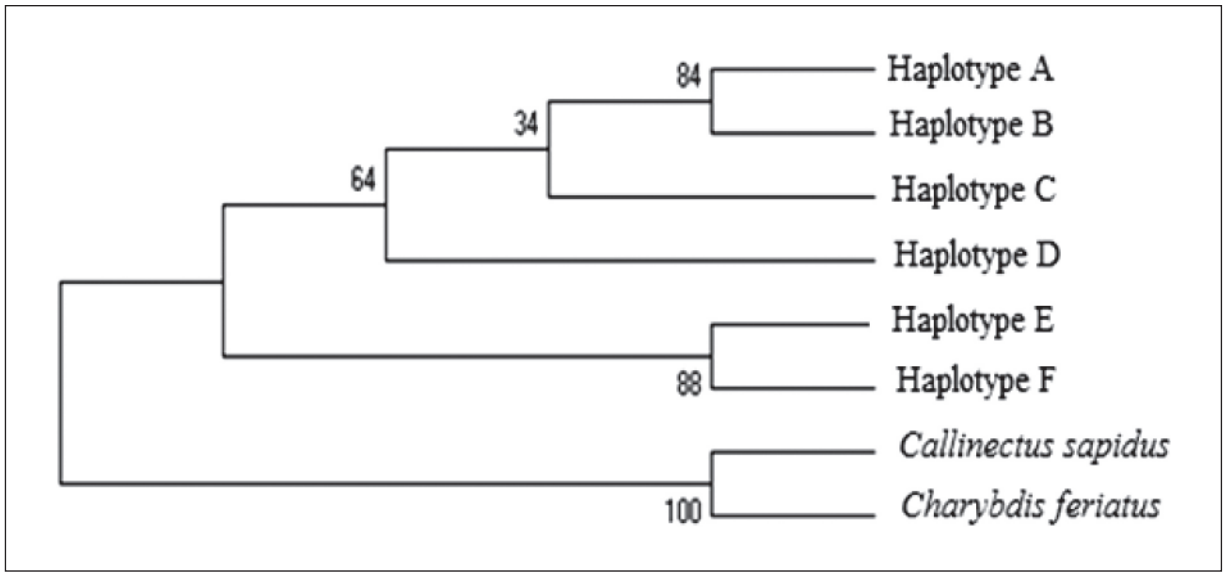

Fig. 1. A Neighbour-Joining (NJ) tree for *P. pelagicus* on two population and outgroups (*C. sapidus* and *C. feriatus*) haplotypes.

Thus, the low level of genetic differentiation and sharing of common haplotype between Teluk Kemang and Batu Pahat suggested that the two populations are genetically similar to each other. Continuous or occasional gene flow might occur among them through free flowing pelagic crab larvae since their geographical distance are near (a distance of ca. 250 km) and within the same ocean current (Strait of Malacca). Similar result was observed in *C. sinense* where high gene flow occurred between population via larval dispersal in the enclosed water body of Tokyo Bay suggesting low genetic differentiation was apparent among discrete local populations and may be adaptive for maintaining their small population as a regional metapopulation (Yuhara *et al.,* 2014).

In conclusion, the high genetic similarities observed between *P. pelagicus* from Port Dickson and Batu Pahat populations obtained in this study concluded that they belong to the same gene pool and can be regarded as a single broodstock population for aquaculture development and sustainable management purposes.

ACKNOWLEDGMENTS

The authors would like to thank the Fish Genetic Laboratory, Dept. of Aquaculture, Universiti Putra Malaysia for providing funding (GP-IPB/2014/9440401), facilities and assistance for the study. The authors would also like to thank Mr. Anuar and Mr. Yusof, staffs of Centre of Marine Science (CoMAS) for their assistance in sample collection in Teluk Kemang, Port Dickson and Batu Pahat, Johor.

REFERENCES

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **7**: 1193-1204.

Esa, Y., Siraj, S.S., Daud, S.K., Ryan, J.R.J., Khairuladha, A.R. & Tan, S.G. 2008. Molecular systematic of mahseers (Cyprinidae) in Malaysia inferred from sequencing of a mitochondrial cytchrome C oxidase, (COI) gene. *Pertanika Journal of Tropical Agricultural Science*, **31**: 263-269.

Kailola, P.J., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A. & Grieve, C. 1993. Australian Fisheries Resources. Bureau of Resource Sciences and Fisheries Research Development Corporation. Canberra, Australia. 422 pp.

Kamarudin, K.R. & Esa, Y.B. 2009. Phylogeny and phylogeography of *Barbonymus schwanenfeldii* (Cyprinidae) from Malaysia inferred using partial cyctochrome b mtDNA gene. *Journal of Tropical Biological Conservation*, **5**: 1-3.

Kessing, B., Croom, H., Martin, A., McIntosh, C., OwenMcMillan, W. & Palumbi, S. 1989. The simple tool's guide to PCR, version 1.0. Department of Zoology, University of Hawaii, Honolulu, HI.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**: 111-120.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., Mcwilliam, H., Valentin, F., Wallace, C.M., Wilm, A. & Lopez, R. 2007. Clustal W and clustal X version 2.0. *Bioinformatics*, **23**: 2947-2948.

Luo, A., Zhang, A., Ho, S.Y., Xu, W. & Zhang, Y. 2011. Potential efficacy of mitochondrial genes for animal DNA barcording : A case study using eutherian mammals. *BMC Genomics,* **12**: 10.1186/1471-2164-12-84.

Maheswarudu, G., Josileen, J., Nair, K., Manmadhan, R. & Arputharaj, M.R. 2008. Evaluation of the seed production and grow out culture of blue swimming crab *Portunus pelagicus* Linnaeus, 1758) in India. *Indian Journal of Marine Sciences*, **37**: 313-321.

Naiyanetr, P. 1998. Checklist of the Crustacean Fauna in Thailand (Decapoda and Stomatopoda). Office of environmental policy and planning biodiversity series 5, 161 pp

Nazia, A.K., Suzana, M., Azhar, H., Nguyen, T.T.T. & Azizah, M.N.S. 2010. No genetic differentiation between geographical isolated populations of *Clarias maceocephalus* (Gunthet) in Malaysia revealed by sequences of mtDNA cytochrome b and D-loop gene regions. *Journal of Applied Ichtyology*, **26**: 568-570.

Ponzoni, R.W., Khaw, H.L., Nguyen, N.H. & Hamzah, A. 2010. Low genetic diversity and population structure of the narrownose shark (*Mustelus schmitti*). *Fish Respiration*, **106**: 468-473.

Romano, N. & Zeng, C. 2006. The effects of salinity on the survival, growth and heamolymph osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. *Aquaculture*, **260**: 151-162.

Saitou, N. & Nei, M. 1987. The neighbour-joining method: A new method for reconstructing phylogenic trees. *Molecular Biology and Evolution*, **4**: 406-425.

Sumpton, W. D., Smith, G. S. & Potter, M. A. 1989. Notes on the biology of the Portunid crab, *Portunus sanguinolentus* (Herbst), in subtropical Queensland waters. *Australian Journal of Marine and Freshwater Research*, **40**: 711-717.

Yuhara, T., Kawane, M. & Furota, T. 2014. Genetic population structure of local populations of the endangered saltmarsh sesarmid crab *Clistocoeloma sinense* in Japan. *PLoS ONE.* **9(1)**: e84720.