Establishment and Operation of a Regional System of Fisheries Refugia in the South China Sea and the Gulf of Thailand

REPORT

GENETIC ANALYSIS OF UROTEUTHIS CHINENSIS FROM BANGKA-BELITUNG WATERS BY RAPD MARKERS

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Method

Genetic samples were acquired from squid tissue (U. chinensis) and preserved under 95% EtOH for further analysis. These samples were taken at 6 sampling points located in A (n = 10), B (n = 8), C (n = 10), D (n = 9), E (n = 10), and F (n = 8) (Fig. 1). The DNA extraction was carried out using gSYNC DNA extraction kit (Geneaid) according to the manufacturing protocol by slightly modifying the final elusion buffer by using 1× TE buffer and incubation time for 5 h at 65℃. The extracted gDNA were diluted 5:95 in ddH2O and then used for PCR amplification.

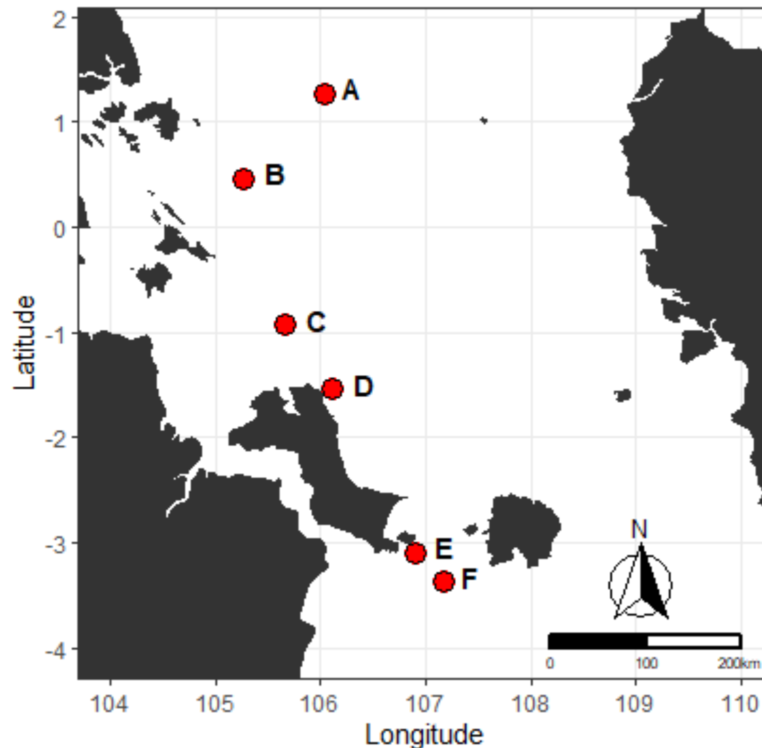

Figure 1. 6 Sampling point of squid (U. chinensis) in Bangka-Belitung waters**.**

Initially, DNA amplification was performed using a set of primers, R1-R8 (Sands et al., 2003). During the amplification optimization, several representatives samples were tested to be amplified using these eight primers under various temperature gradients. The temperature was set 2 points below or above the melting temperature. From the optimization process, we found primers R5, R6, and R8 to be the most suitable primers for use in the analysis, taking into account the quality of the amplification result. gDNA from 55 samples were amplified using these three primers. Thermocycling process was performed for pre-denaturation at 94℃ for 2 min, 40 cycles of denaturation at 94℃ for 15 s, annealing at 35℃ for 60 s, elongation at 72℃ for 90 s, and final elongation at 72℃ for 5 min. The PCR reagent consist of 6,25 µL MyTaqHS-Redmix (Bioline), 0,5 µL primer, 5,25 µL ddH2O, and 0,5 µL gDNA. The amplification product was then separated under 1% agarose gel in 1×TAE for 30 min at 100 V. The electropherogram was visualized under UV lighting using the UVP gel doc system.

The resulting electropherogram was used for scoring with the aid of GelAnalyzer19.1 and to generate binary data showing the DNA band polymorphism of each sample analyzed. The binary data were analyzed with Popgen (Yeh, 1999) and StAMP (Parks et al., 2014) to determine genetic variation, genetic distance, and pairwise-Fst, adegenet for PCoA (Jombart, 2008), and STRUCTURE for admixture analysis (Pritchard et al., 2000). To confirm genotyping that resulted from RAPD

markers, the phylogenetic (RAxML) tree was constructed using 29 16s rRNA sequence accession from China (HQ529571-HQ529575, EU349467-EU349480) and Australia (MK030621-MK030775).

Result

Molecular studies for several species of squid were still focused on efforts to characterize nucleotides variation using partial gene targeting to elaborate species identity (Shin et al., 2009; Wen et al., 2017). In this study, a genetic analysis using RAPD (Randomly amplified polymorphic DNA) was carried out. The use of RAPD markers for genetic studies in several cephalopods has been reported to be successful in identifying genetic differentiation at the population level (Ibanez et al., 2011; Sandoval-Castellanos et al, 2007; Sands et al., 2003). By considering the limited DNA sequence available for U. chinensis from karimata strait, a preliminary study using available reference sequence from genebank was hard to conduct. This preliminary study was usually important to screen the best marker and molecular technique for genotyping either by sequence data or DNA fingerprinting technique (RFLP, T-RFLP, etc). However, the use of RAPD marker was viewed as the most potential alternative marker, supported by its ability to identify polymorphic alleles and provide a genetic characteristic of the species being analyzed. This study was successfully demonstrated the U. chinensis amplification product from three RAPD markers, and the resulting 7-10 bands (Fig. 2)

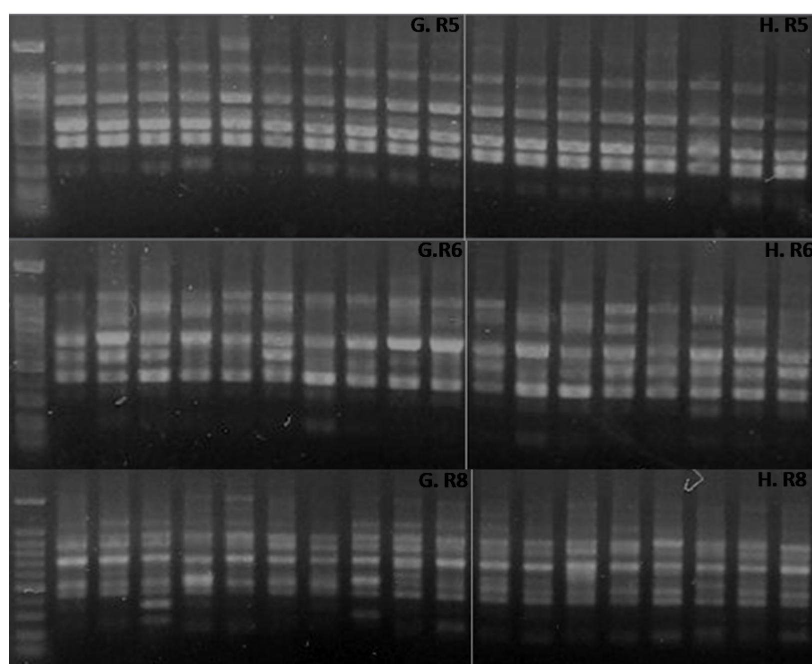

Figure 2. Electropherogram of amplified DNA using markers R5, R6, and R8 from populations G and H.

Table 1. Sample size (N), Observed allele (Na), Genetic variation (H), Shanon information index (I), Number of the polymorphic locus (nPol), and percentage of the polymorphic locus (%Pol) from all U. chinensis population.

Pop	N	Na	H	I	nPol	%Pol
A	10	1.50	0.15	0.24	14	50.00%
B	8	1.53	0.21	0.31	15	53.57%

C	10	1.53	0.18	0.27	15	53.57%
D	9	1.89	0.27	0.27	25	89.29%
E	10	1.64	0.21	0.32	18	64.29%
F	8	1.53	0.19	0.29	15	53.57%

From the data obtained, it is known that the genetic variation in each population is between 0.15 to 0.27. The highest genetic variation was identified in the sample from population D (H = 0.27). Population D in this study is a population of genetic characteristics that is quite diverse genetically by considering that this population has almost 90% of alleles that are polymorphic compared to other populations that are relatively lower and between 50% - 65%. The number of observed alleles in population D also had the highest value (Na = 1.89) compared to other populations which ranged from 1.5-1.6. This information indicates that population D has better genetic quality than other locations.

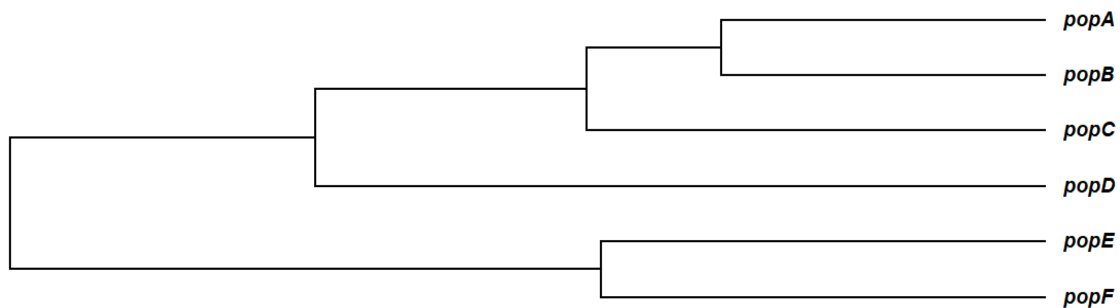

Figure 3. Genetic distance dendrogram among all population

Table 2. U.chinensis genetic distance from 6 population analyzed

Pop	**A**	**B**	**C**	**D**	**E**	**F**
A						
B	0.043					
C	0.050	0.059				
D	0.098	0.052	0.099			
E	0.101	0.068	0.119	0.123		
F	0.107	0.106	0.113	0.139	0.056	

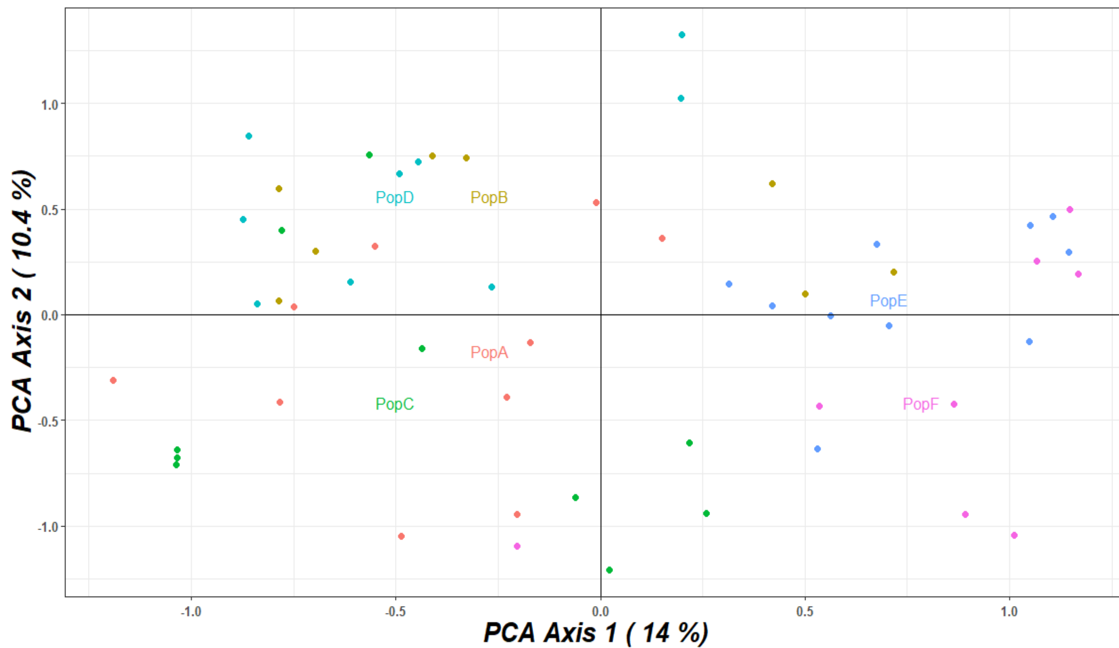

Figure 4. Principal Coordinate Analysis (PCoA) based on RAPD data

The genetic distance in each observed squid population showed the formation of two main clades, the first clade is a cluster of populations A-D, and the second clade is a group of populations E and F (Fig. 3). The genetic distance among the squid population in the waters of Bangka Belitung shows a relatively low genetic distance (D<0.2) with a genetic distance value ranging from 0.04 to 0.139 (Table 2). This relatively low genetic distance suggests that there is a genetic mixture among the observed populations. This is also confirmed by the results of the Principal Coordinate Analysis which shows the distribution of the ordinate points of each sample in the entire observed population (Fig. 4). The ordinate points of populations E and F in Fig. 4 tend to be clustered in quadrant 2, indicating these two populations have similar alleles. However, populations A, B, C, and D have a wide distribution of ordinate points. These results show that the squid population in the second clade (E and F) tends to keep only part of the alleles.

With the high genetic mixture, especially from the allele contribution of the A-D population as identified from the PCoA results, genetic differentiation at a relatively low population scale may indicate contact and interbreeding between individuals in these different populations. This assumption is supported by the results of the calculation of the pairwise-Fst value in the entire observed population below 0.15 (Table 3). The low pairwise-Fst value proves that there is no differentiation of squid populations in the waters of Bangka Belitung. This is also supported by the results of the admixture analysis at K = 2, which does not show a structured population pattern of the six squid populations in the waters of Bangka Belitung (Fig. 5).

Table 3. Pairwise-Fst calculated from RAPD data on U. chinensis genetic sample

Populasi	A	B	C	D	E	F
A						
B	0.000					
C	0.000	0.000				

D	0.082	0.000	0.072			
E	0.124	0.000	0.146	0.125		
F	0.125	0.037	0.117	0.141	0.000	

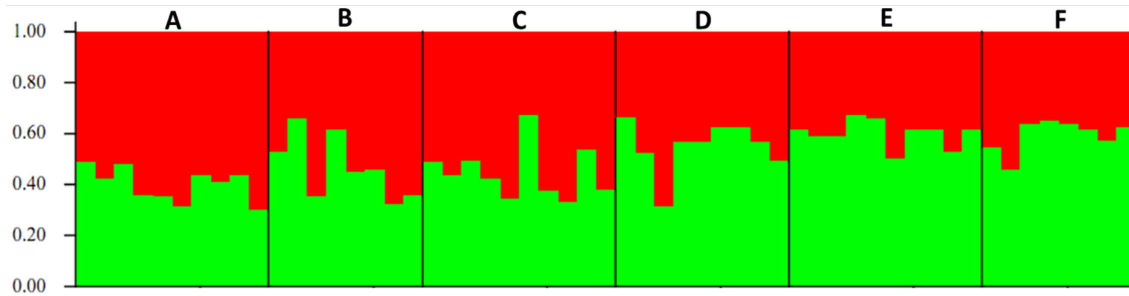

Figure 5. Admixture-bar plot showed population structure from 6 U. chinensis sampling point

The low genetic differentiation evidence among squid populations in Bangka-Belitung waters is also supported by U. chinensis phylogenetic trees from several countries. In the phylogenetic tree constructed using 16S gene DNA sequences, it is known that all of the U. chinensis sequence data forming a monophyletic clade which shows a close genetic relationship and does not show any new clade that correlates with geographic distance between populations (Fig. 6).

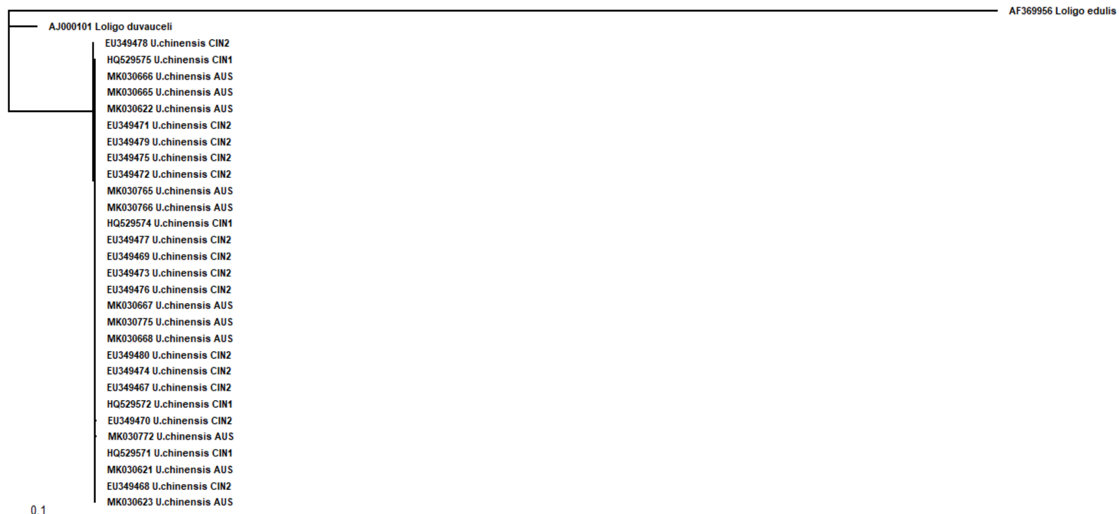

Figure 6. Phylogenetic tree from 16s rRNA U. chinensis 29 sequence accession from China (CIN) and Australia (AUS).

References

Ibáñez, C. M., Cubillos, L. A., Tafur, R., Argüelles, J., Yamashiro, C., & Poulin, E. (2011). Genetic diversity and demographic history of Dosidicus gigas (Cephalopoda: Ommastrephidae) in the Humboldt Current System. Marine Ecology Progress Series, 431, 163-171.

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24(11), 1403-1405.

Parks, D. H., Tyson, G. W., Hugenholtz, P., & Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics, 30(21), 3123-3124.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945-959.

Sandoval-Castellanos, E., Uribe-Alcocer, M., & Díaz-Jaimes, P. (2007). Population genetic structure of jumbo squid (Dosidicus gigas) evaluated by RAPD analysis. Fisheries Research, 83(1), 113-118.

Sands, C. J., Jarman, S. N., & Jackson, G. D. (2003). Genetic differentiation in the squid Moroteuthis ingens inferred from RAPD analysis. Polar Biology, 26(3), 166-170.

Sin, Y. W., Yau, C., & Chu, K. H. (2009). Morphological and genetic differentiation of two loliginid squids, Uroteuthis (Photololigo) chinensis and Uroteuthis (Photololigo) edulis (Cephalopoda: Loliginidae), in Asia. Journal of Experimental Marine Biology and Ecology, 369(1), 22-30.

Wen, J., Tinacci, L., Acutis, P. L., Riina, M. V., Xu, Y., Zeng, L., & Armani, A. (2017). An insight into the Chinese traditional seafood market: species characterization of cephalopod products by DNA barcoding and phylogenetic analysis using COI and 16SrRNA genes. Food Control, 82, 333-342.

Yeh, F. (1999). POPGENE 32v. 1.31 Microsoft Window-based freeware for population genetic analysis. ftp://ftp. microsoft. com/Softlib/MSLFILES/HPGL. EXE.