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POPULATION GENETICS OF LONGTAIL TUNA *(Thunnus tonggol)* (BLEEKER, 1851) FROM EAST MALAYSIA BASED ON MITOCHONDRIAL DNA D-LOOP MARKER

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Abstract: Present study investigates the genetic diversity and genetic distribution of the longtail tuna *Thunnus tonggol* collected from east Malaysia (Borneo states of Sabah and Sarawak) based on mitochondrial DNA D-loop sequence analysis. 58 fish samples were obtained, specifically from Kota Kinabalu, KK (n = 22), Miri, MR (n=20) and Bintulu, BT (n = 17). DNA template was isolated using the salt extraction method. Final length of 404 base pair (bp) D-loop sequences revealed 52 haplotypes that comprise of 77 variable sites (38 of parsimony informative and 39 singleton). A total of 20 haplotypes were found in KK, 19 haplotypes in MR and 16 haplotypes in BT. Molecular diversity indices revealed high haplotype diversity and low nucleotide diversity in all populations; KK ($h = 0.9913 \pm 0.0165$, $\pi = 0.00239 \pm 0.0127$), MR ($h = 0.9942 \pm 0.0193$, $\pi = 0.0226 \pm 0.0121$) and BT ($h = 0.9926 \pm 0.0230$, $\pi = 0.0196 \pm 0.0171$). Population comparison pairwise F_{ST} show that KK and BT were significantly genetically differentiated. The result from this study will be beneficial for fisheries management and also to provide information on the population genetics of *T. tonggol* in East Malaysian waters.

Keywords: *Thunnus tonggol*, mitochondrial DNA D-loop, population structure, genetic diversity, fisheries management

Introduction

The longtail tuna *Thunnus tonggol* is an economically important pelagic-neritic species in Malaysia. This species is commonly found in tropical to temperate habitats along the Indo-West Pacific region. Compared with other *Thunnus* species, longtail tuna is smaller in size with maximum length of 145 cm, also tend to form fast-moving and small schooling (Griffiths, 2010). Global catches of longtail tuna increased largely to around 100,000 tan per year in 1985 and continued to escalated to 248,000 tan in 2007 (Griffiths, 2010). A minimum of 17 countries within the Indo-Pacific have heavily exploited the longtail tuna by small scale and artisanal fisheries as a result from their coastal distribution (Griffiths, 2010). In Malaysia specifically in Borneo states of Sabah and Sarawak, the catch amount of tuna were just a small number; however in 1991 and 1992 these states recorded the highest tuna catch among other states in Malaysia. The total landing of the species were 13,000 metric tons (mt) and 19,000 mt for both states in the year 1991 and 1992 respectively (Griffiths, 2010).

Despite the increasing demand for the *Thunnus tonggol* and heavily exploited by the purse seine and gillnet catch industry (Griffith et al., 2009) due to its high economic value, limited studies on its population genetics were conducted especially around Malaysian waters. Previous studies on the population genetics were

only recorded at the Indian Ocean, Australia waters and Wallace's Line (Kunal *et al.,* 2014; Kumar & Kocour, 2015; Willette *et al.,* 2016). Understanding the genetic diversity and fisheries stock structure are crucial for the sustainable long-term fisheries management. Study about population genetics of the longtail tuna will be beneficial for fisheries management in the future especially in the east Malaysia.

The mitochondrial DNA (mtDNA) is broadly used as a tool for molecular genetic studies due to its compact size (16–17 kbp), high percentage for maternal inheritance and high rate of mutation. MtDNA is also more sensitive to demographic events that may have affected genetic variation, such as reduction in population size and geographic isolation (Durand et al, 2005). The control region of mtDNA, also known as the D-Loop, is known as the only non-coding division in the vertebrate mitochondrial genome (Shui *et al*., 2008). Several studies have shown that the variation of DNA sequence in this region is relatively high (Zhang *et al.,* 2016; Lalitha and Chandawvar), making it as one of the best markers for genetic study. Though it contains highly conserved sequences (Saccone et al., 1987), this region is known to exhibit some of the highest rates of evolution in the mtDNA.

The present study involved the populations of longtail tuna obtained from the east Malaysia, and their population genetics were assessed based on the mtDNA D-loop region. The specific objectives in this study were to investigate the genetic diversity and population structure of longtail tuna in the East Malaysia waters based on mtDNA D-loop marker.

Materials and Methods

Tissue Samples collection and DNA extraction

Random samples of longtail tuna, *Thunnus tonggol* were collected from East Malaysia which cover Kota Kinabalu (KK) in Sabah, Miri (MR) and Bintulu (BT) in Sarawak (Figure 1, Table 1). Small portion of the fin tissue were cut from either the dorsal or caudal fin and each of the fins was preserved in a 1.5 ml microcentrifuge tube containing absolute ethanol until subsequent use. Genomic DNA was isolated by using the salt extraction method (Miller *et al.,* 1988) and the presence of DNA was checked with 0.7% agarose gel electrophoresis.

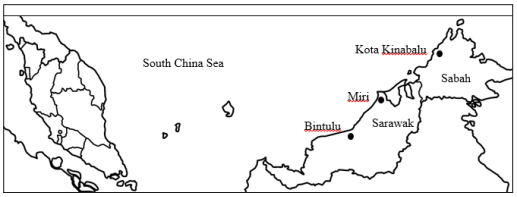

Figure 1: Location of samples landed marked by black cross

Table 1: Sampling information of *T. tonggol* including landed location, date of collection and sample size

Population	Coordinates	Collection date	Sample size
Kota Kinabalu (KK)	5°58'59.4"N 116°04'22.5"E	19/7/2017	22
Miri (MR)	4°23'31.1"N 113°59'07.6"E	7/3/2018	19
Bintulu (BT)	3°10'13.8"N 113°02'25.8"E	8/3/2018	17

Polymerase chain reaction (PCR) optimization

PCR amplification of mtDNA D-loop was using primer pair Pro889U20 (5′-CCW CTA ACT CCC AAA GCT AG-3′, forward) and TDKD1291L21 (5′-CCT GAA ATA GGA ACC AAA TGC-3′, reverse). The reaction mixture consist of 50-100 ng of genomic DNA, 0.6 µM of each primer, 0.2 mM of dNTP (iNtRON), 1x PCR buffer (iNtRON), 4.2 mM $MgCl_2$ (iNtRON) and 0.08 U of *Taq* polymerase (iNtRON). The PCR amplification were performed in a thermal cycler under the conditions of initial 1 minute (min) denaturation at 98 ˚C, followed by 1 min of 35 cycles at 95 ˚C, annealing for 1 min at 55 ˚C, 2 min at 72 ˚C for extension and a final extension at 72 ˚C for 1 min before termination of the reaction at 10 ˚C. Presence of

PCR products were confirmed on 1.7% agarose gels stained with SyBr Safe. PCR products were then sent for direct sequencing at First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) using the same primer.

Data analysis

All sequences were aligned and edited using ClustalW implemented in MEGA X (Kumar *et al.,* 2018). The aligned dataset were analyzed for the variable nucleotide sites, parsimony informative sites, number of haplotypes and nucleotide diversity in MEGA X. Haplotype or gene diversity (h) and nucleotide diversity (π) were estimated to infer the DNA polymorphism at each sampling location using Arlequin 3.5 (Excoffier & Lischer, 2010). The haplotype diversity (h) measures the probability of uniqueness of a haplotype in each population, while the nucleotide diversity (π) is the mean number of pairwise nucleotide differences among individuals in a sample. Based on the analysis of molecular variance (AMOVA), relative genetic differentiation between populations were determined by using the population pairwise comparison statistic, F_{ST}. Arlequin 3.5 was used to calculate the significance or differences among populations and spatial population structuring. Phylogenetic tree of haplotypes *T. tonggol* were reconstructed using neighbor-joining (NJ) method based on the Kimura 2-P model in the MEGA X. *Schizopygopsis pylzovi* was used as outgroup in the D-loop sequences obtained from GenBank (KY461281).

Results and Discussion

Genetic diversity

The partial mtDNA sequences of D-loop region were obtained from 58 individuals with final length at 404 base pair (bp). Seventy-seven (19%) variable sites were retrieved including 38 parsimony informative sites and 39 singleton. The sequences revealed 52 haplotypes among the three populations. Haplotype 20 and haplotype 46 were present in both populations, MR and BT. Three haplotypes were found in two locations; two were respectively in MR and BT, one in KK and BT. There were 20 haplotypes found in KK, 19 haplotypes in MR and 16 haplotypes in BT (Table 2). All populations had high haplotype diversity (0.9913-0.9942) and nucleotide diversity (0.0196-0.0239). Nucleotide substitution model from this sequence was (T92 + G) by checking in the Mega X for best nucleotide substitution model. Polymorphic sites accessed for each population were 52 from KK, 42 from MR and 31 from BT (Table 2).

Table 2: Genetic diversity measurement of *T.tonggol* based on D-Loop sequences.

Population	N	H	S	h ± SD	π ± SD
KK, Sabah	22	20	52	0.9913 ± 0.0165	0.0239 ± 0.0127
MR, Sarawak	19	19	42	0.9942 ± 0.0193	0.0226 ± 0.0121
BT, Sarawak	17	16	31	0.9926 ± 0.0230	0.0196 ± 0.0107

N, sample size; H, number of haplotypes; S, number of polymorphic sites; h, gene diversity; π, nucleotide diversity; SD, standard deviation

Genetic diversity has the potential to give impact to a larger area of population, community and ecosystem whether it is done by directly or indirectly (Hughes *et al*., 2008). In general, genetic diversity of a species is commonly known for its adjustable and mutative potential. When a species showed their abundance of genetic diversity, this suggest that it has a high possibility to adapt to a certain area, high mutative potential and had genetic development for a species. On the other hand, if the species has a poor genetic diversity it can give side effect to the population and performance of genetic resources (Guo *et al*., 2014). Usually it is suggested that when a population went through a serious barrier, the population will experience an enormous destruction of its genetic variability (Qi *et al*., 2007).

In this study, *Thunnus tonggol* illustrated a high h value (0.9913-0.9942) across all populations and high nucleotide diversity (0.0196-0.0239) based on D-Loop marker, which is similar to other studies reported for other *Thunnus* species. Akbar *et al*. (2018) and Martinez *et al*. (2006) revealed that bigeye tuna (*T. obesus*) from the Atlantic Ocean recorded the h value 1.00 and nucleotide diversity was 0.031 based on D-Loop marker. Another study of the bigeye tuna from Western Pacific Ocean indicate that the high value of h (0.999) and nucleotide diversity (0.043) (Chiang *et al*., 2008). Migration of fish might become the factor for differences in genetic diversity besides from the mixing of individuals between other populations. According to Wild (1994) the rate of migration of wild tuna are higher than other marine fish that led to a greater possibilities of mixed genetic materials with other populations. When a population has high genetic diversity, it probably has a better chance of survival due to each gene in population that has a different response to environmental conditions (Yusron, 2005).

Genetic structure

Through the analysis of molecular variance (AMOVA), it shows that *T. tonggol* from East Malaysia waters contribute a highly significant percentage of variations within populations (97.64%) and only 2.36% of the variations was attributed to differences among populations (Table 3). This was evidence to a high percentage of genetic diversity in each population. The percentage of variations among populations was negligible perhaps caused by the high gene flow in the populations in Malaysia waters. Gene flow between the populations could be the factor for the closeness of the genetics (Akbar *et al*., 2018). High ability to spread and strong scattering capacity could increase the genetic interchange among populations across their region which could be a potential reasons for the low genetic differentiation among populations (Guo *et al*., 2014).

Table 3: Comparison of population samples

Source of variation	d.f	SS	Variance components	Percentage of variation
Among populations	2	13.130	0.10835 Va	2.36
Within populations	55	246.508	4.48196 Vb	97.64
Total	57	259.638	4.59032	

d.f, degree of freedom; SS, sum of squares

Population pairwise F_{ST} values ranged from 0.0064 (KK-MR) to 0.0678 (KK-BT) (Table 4). This revealed that population from KK was significantly genetically different from BT ($P < 0.05$). Genetic distance between KK and MR was 0.0283, KK and BT were 0.0241 and MR and BT was 0.0217 (Table 5), of which all pair of genetic distance comparisons were relatively low based on the D-loop sequences. The phylogenetic tree of the haplotypes *T. tonggol* was reconstructed using neighbor-joining (NJ) approach with Kimura 2-P model (Figure not shown). One individual was included as the outgroup which is *Schizopygopsis pylzovi*. The topology built from the haplotypes was shallow with some branches and clusters of samples. The tree showed a weakly associated of the samples among the three populations.

Table 4: Pairwise F_{ST} value

Population	KK	MR	BT
KK			
MR	0.0064		
BT	0.0678*	-0.0092	

Note:*Significant $P < 0.05$

Table 5: Genetic distance of the three populations

	KK	MR	BT
KK			
MR	0.0238		
BT	0.0241	0.0217	

KK: Kota Kinabalu; MR: Miri; BT: Bintulu

For the population KK-BT there was a significantly difference in the structure between the two populations, while there was no significantly genetically difference in the gene structure of populations from MR-BT and MR-KK. In mtDNA studies of migratory pelagic species the genetic population structure is usually different than the others. However, patterns of panmixia are often published, as well as other pelagic *Thunnus* relatives such as the bigeye tuna, *T. obesus* and yellowfin tuna, *T. albacores* (Durand *et al*., 2005; Chiang *et al*., 2006).

Chiang *et al*., (2006) reported that absent of genetic structure indicated that there are wide-range of genomics outflow within the Indo-Pacific Ocean compared to Atlantic Ocean. There has been a report regarding analysis of another tuna species, for example, population genetic studies on mtDNA of Pacific yellowfin revealed a very low levels of genetic differentiation (Appleyard *et al*., 2001 and Ely *et al*., 2005). Genetic distances between three populations were almost at the same level which revealed that genetic differentiation is low. The geographical position between three populations which is not very far apart only 531 km increase the chance of genetic similarity (Akbar *et al*., 2018).

Conclusion

We used mitochondrial DNA D-loop sequences to assess the genetic structure of *Thunnus tonggol* from three populations in East Malaysia. Populations of *T. tonggol* showed high haplotype diversity and highly significant variation within populations where 97.4% of the genetic variation was contributed within population. KK-BT revealed they were significantly difference to each other. To avoid the decrease of genetic diversity, a better fishery management is required to keep the stock away from any disturbance. Authorities should carried out management strategies at local Malaysia waters such as to forbid fishing activities during breeding season which usually takes place from May to June, increasing mesh size of fishing gears and lastly to control exotic fishes from being heavily exploited (Akbar *et al*., 2018) in order to maintain the population of marine species. Besides, protection to the habitats from human activities or water pollution must be increased and basic laws for conservations of *Thunnus tonggol* in East Malaysia waters ought to be enhanced. This research has contributed various information about population genetics of longtail tuna and provided a scientific ground for the assessment of genetic data bases and resource management of this species. Further investigation on the *Thunnus tonggol* involve inclusion of more samples from other geographical areas and may consider other molecular markers such as microsatellites.

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References

Akbar, N., Irfan, M., & Aris, M. (2018). Population Genetics and Phylogeography of Bigeye Tuna in Moluccas Seas, Indonesia. *Indonesian Journal of Marine Sciences Ilmu Kelautan*, *23*(4), 145-155.

Appleyard, S. A., Grewe, P. M., Innes, B. H., & Ward, R. D. (2001). Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. *Marine Biology*, *139*, 383–393.

Chiang, H. C., Hsu, C. C., Lin, H. D., Mac, G. C., Chiang, T. Y., & Yang, H. Y. (2006). Population structure of bigeye tuna (*Thunnus obesus*) in the South China Sea, Philippine Sea and western Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research*, *79*(1-2), 219–225.

Durand, J. D., Collet, A., Chow, S., Guinand, B., & Borsa, P. (2005). Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. *Marine Biology*, *147*(2), 313–322.

Ely, B., Vinas, J., Bremer, J. R. A., Black, D., Lucas, L., Covello, K., Labrie, A.V., & Thelen, E. (2005). Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). *BMC Evolution Biology*, *5*(1), 19.

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. *10*(3), 564-567.

Griffiths, S. P., Fry, G. C., Manson, F. J., & Lou, D. C. (2009). Age and growth of longtail tuna (Thunnus tonggol) in tropical and temperate waters of the central Indo-Pacific. *ICES Journal of Marine Science*, *67*, 125–134.

Griffiths, S, P. (2010). Stock assessment and efficacy of size limits on longtail tuna (*Thunnus tonggol*) caught in Australian waters. *Fisheries Research, 102*(3), 248-257.

Guo, S. S., Zhang, G. R., Guo, X. Z., Wei, K. J., Yang, R. B., & Wei, Q. W. (2014). Genetic diversity and population structure of *Schizopygopsis younghusbandi* Regan in the Yarlung Tsangpo River inferred from mitochondrial DNA sequence analysis. *Biochemical Systematics and Ecology*, *57*, 141-151.

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609-623.

Kumar, G., & Kocour, M. (2015). Population genetic structure of tunas inferred from molecular markers: a review. *Reviews in Fisheries Science & Aquaculture*, *23*, 72-89.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platform. *Molecular Biology and Evolution, 35,* 1547-1549.

Kunal, S. P. Kumar, G., Menezes, M. R., & Meena, R. M. (2014). Genetic homogeneity in longtail tuna *Thunnus tonggol* (Bleeker, 1851) from the northwest coast of India inferred from direct sequencing analysis of the mitochondrial DNA D-loop region. *Marine Biology Research, 10*(7), 738-743.

Lalitha, R., & Chandavar, V. R. (2018). Intraspecific variations in Cyt b and D-loop sequences of Testudine species, *Lissenys puncata* from south Karnataka. *Journal of Advanced Research, 9*, 87-95.

Martinez, P., Gonzales, G. E., Castilho, R., & Zardoya, R. (2006). Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). *Molecular phylogenetics and evolution*, *39*(2), 404–416.

Saccone, C., Attimonelli, M., & Sabisa, E., (1987). Structural elements highly preserved during the evolution of the D-loop containing region in vertebrate mitochondrial DNA. *Journal of Molecular Evolution*, *26*, 205-211.

Shui, B. N., Han, Z. Q., Gao, T. X., & Miao, Z. Q. (2008). Genetic structure of Japanese Spanish mackerel (*Scomberomorus niphonius*) in the East China Sea and Yellow Sea inferred from AFLP data. *African Journal of Biotechnology*, *7*(21), 3860-3865.

Qi, D. L., Guo, S. C., Zhao, X. Q., Yang, J., & Tang, W. J. (2007). Genetic diversity and historical population structure of Schizopygopsis pylzovi (Teleostei:

Cyprinidae) in the Qinghai-Tibetan Plateau. *Freshwater Biology*, *52*(6), 1090-1104.

Wild, A. (1994). A review of the biology and fisheries for yellowfin tuna, *Thunnus albacares* in the eastern Pacific Ocean. *FAO Fisheries Technical Paper*, *336*, 52-107.

Willette, D. A., Santos, M. D., & Leadbitter D. (2016). Longtail tuna *Thunnus tonggol* (Bleeker, 1851) shows genetic partitioning aross, but not within, basins of the IndoPacific based on mitochondrial DNA. *Journal of Applied Ichthyology, 32*, 318-323.

Yusron, E., (2005). Utilization of genetic diversity in marine biological resource management. *Oceana Journal*, *30*(2), 29–34.

Zhang, J. J., Duan, J. R., zhou, Y. F., Peng, J. Y., & Fang, D. A. (2016). Genetic diversity of mitochondrial control region (D-loop) polymorphisms in Coilia ectenes taihuensis inhabiting Taihu Lake, China. *Genetics*