UNIVERSITI PUTRA MALAYSIA

POPULATION GENETIC STRUCTURE OF Eleutheronema tetradactylum(Shaw, 1804) AND Eleutheronema rhadinum (Jordan and Evermann, 1902) IN MALAYSIAN WATERS

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By

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Abstract of the thesis prepared to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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November 2019

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The four finger threadfin, *Eleutheronema tetradactylum* (Shaw, 1804) and East Asian four finger threadfin, *Eleutheronema rhadinum* (Jordan and Everman, 1902) are two species of the Polynemidae family that can be found in Malaysian waters. Common name among local is Senangin. There have been no population genetic studies in both species in Malaysia, which are important to provide genetic information for sustainable fisheries management of the two species. Moreover, both Senangin species are also difficult to distinguish morphologically, thus their taxonomic classification are not fully resolved. Therefore, the aims for this study were to determine the levels of genetic divergences between the two Senangin fishes, and to quantify their population genetic structure by using both mitochondrial DNA and microsatellite markers. Totally 151 samples of Senangin were collected from six location around Malaysian coastal water. Samples were obtained from commercial fishing activities. Among 151 individuals, 20 distinct haplotypes were defined. The phylogenetic tree (maximum likelihood) showed two genealogical branches were recognized among the haplotype, this also proven in pairwise distance among the haplotypes. Level of genetic divergence was substantially high (15-17%) supported their taxonomic status as distinct species. The nucleotide diversity was low in *E. tetradactylum* and *E. rhadinum*. Both species show the sharing of haplotypes between different populations and this support that both species had a historically widespread natural distribution in the region in the past. Microsatellites analyses for *E. tetradactylum* also indicated low levels of genetic variation and high degree of population structure among the *E. tetradactylum* populations. The average observed heterozygosity (H_O = 0.5191) obtained was lower than the standard heterozygosity found in most marine populations (H_O = 0.79). Through assignment test all samples were assigned to their respective populations. The low genetic variation in both of mitochondrial DNA and microsatellite proved the evidence of over-exploitation of *E. tetradactylum* in Malaysia. In conclusion, the present study was able to differentiate between *E. tetradactylum and E. rhadinum* using mitochondrial gene,

Cytochrome Oxidase I. The levels of genetic differentiation and population structure between the two species were successfully quantified.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

STRUKTUR GENETIK POPULASI *Eleutheronema tetradactylum* (Shaw 1804) DAN *Eleutheronema rhadinum* (Jordan dan Evermann 1902) DI PERAIRAN MALAYSIA

Oleh

NURUL ATIKAH BINTI WAHID

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Ikan Senangin rambu empat, *Eleutheronema tetradactylum* (Shaw, 1804) dan Senangin rambu empat Asia Timur, *Eleutheronema rhadinum* (Jordan and Everman, 1902) ialah dua spesies dari famili Polynemidae yang boleh dijumpai di perairan Malaysia. Tidak terdapat sebarang kajian berkaitan populasi genetik di Malaysia, yang penting untuk memberikan maklumat genetik untuk pengurusan perikanan yang mampan bagi kedua- dua spesies Senangin. Ikan Senangin ini juga sukar dibezakan melalui morfologi, oleh sebab itu klasifikasi taksonomi tidak dapat diselesaikan sepenuhnya. Oleh itu, tujuan kajian ini ialah untuk menentukan tahap perbezaan genetik antara kedua- dua ikan Senangin, dan mengkuantitikan struktur populasi genetik dengan menggunakan DNA mitokondria dan penanda mikrosatelit. Kesemua 151 sampel ikan Senangin dikumpul dari enam lokasi di sekitar pesisiran pantai Malaysia. Sampel diperolehi daripada aktiviti penangkapan ikan komersial. Antara 151 individu, 20 haplotaip berbeza dikenal pasti. Pokok filogenetik (*Maximum likelihood*) menunjukkan dua cabang genealogi dikenal pasti di antara haplotaip, ini juga terbukti dalam jarak berpasangan antara haplotaip. Tahap perbezaan genetik sangat tinggi (15-17%) menyokong status taksonomi sebagai spesies yang berlainan. Kepelbagaian nukleotida *E. tetradactylum* dan *E. rhadinum* adalah rendah. Kedua- dua spesies menunjukkan perkongsian haplotaip antara populasi berbeza dan ini memberi sokongan bahawa kedua- dua spesies mempunyai taburan semula jadi secara meluas di rantau ini pada masa lalu. Analisis mikrosatelit untuk *E. tetradactylum* juga menunjukkan tahap variasi genetik yang rendah dan tahap struktur populasi yang tinggi antara populasi *E. tetradactylum*. Purata heterozigositi (H_O = 0.5191) yang diperoleh adalah rendah daripada piawai heterozigositi dalam kebanyakan populasi marin (H_O = 0.79). Melalui ujian "*assignment*" kesemua sampel tetap pada populasi masing- masing. Variasi genetik yang rendah pada kedua- dua DNA mitokondria dan mikrosatelit membuktikan eksploitasi yang berlebihan pada *E. teradactylum* di Malaysia. Kesimpulannya, kajian ini dapat membezakan antara *E. tetradactylum* and *E. rhadinum* dengan menggunakan

mitokondria gen, “*sitokrom c oksides I”*. Tahap perbezaan genetic dan struktur populasi antara dua spesies Senanign telah berjaya dianggarkan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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ºC	Degree celcius
m	Meter
cm	Centimeter
mm	Millimeter
mg	Milligram
Mm	Millimolar
ml	Millileter
min	Minute
µl	Microliter
bp	Base pair
kb	Kilo base
rpm	Revolutions per minute
Dntp	Deoxynucleotide triphosphate
V	Volt
DNA	Deoxyribonucleic acid
$MgCI_2$	Magnesium chloride
TBE	Tris/Borate/EDTA
NCBI	National Center for Biotechnology Information
BOLD	Barcode of Life Data
ML	Maximum Likelihood
NJ	Neighbour Joining
h	Hour
mtDNA	Mitochondrial Deoxyribonucleic acid
ITIS	Integrated Taxonomic Information System

CHAPTER 1

INTRODUCTION

Identification of biodiversity of the marine ecosystem is a key point of fisheries management and conservation. Nowadays, marine ecosystems worldwide are under risk with many fish species and populations suffering from human over-exploitation (Chatterjee, 2017). The increasing of genetic drift by over fishing can result the changes in genetic diversity and population structure. Genetic drift by hand migration and gene flow, facilitated due to moving of fish eggs and larvae by passive transport or by active adult migration between different locations, first tend to keep population homogenous, seconds adds genetic variation to population. Thus, there need for improved conservation efforts which also allow sustainable exploitation.

The four finger threadfin, *Eleutheronema tetradactylum* (Shaw, 1840) and East Asian four finger threadfin, *E. rhadinum* (Jordan and Everman, 1902) belongs to the Polynemidae family. This two species has overlapping species distribution pattern. The *E. tetradactylum* can be found along the coasts of South Africa, Australia, Indonesia, Malaysia, Bangladesh, Kuwait, Myanmar, Philippines, Sri Lanka, Vietnam (Motomura, 2002) and China (Wang et al.,2014). The *E. rhadinum* was distributed in East Asia (China, Japan and Vietnam) and generally can be found on continental shelves (Motomura, 2004). This two species especially *E. tetradactylum* is migrating higher up the rivers than other polynemids. Mature *E. tetradactylum* enters into the estuary for breeding once salinity of water starts increasing.

Eleutheronema tetradactylum is a species that have very high commercial and important capture species in Malaysia (Motomura, 2004). Local price for large fish (40-50cm total length) is around RM 22.00 to RM 26.00. *Eleutheronema rhadinum* has little commercial important compare to *E. tetradactylum* because *E. rhadinum* population always has been taken with *E. tetradactylum* (Motomura et al., 2002). They are actually two different species but have been classified as one recognized species (Motomura, 2004). This because its share a large degree of overlap. Thus, it is important to know the stock barrier to an enhance approach that integrates molecular data into management and conservation goals.

There are some of previous studies on polynemid fish stock structures using non molecular markers (Zischke et al., 2009; Moore et al., 2011; Newman et al., 2011). The parasites and conventional tagging was used to determine the stock structure of four finger threadfin *E. tetradactylum* at the East Queensland Coast (Zischke et al., 2009) and northern Australia (Moore et al., 2011). A stable isotope in sagittal otolith carbonates were utilizes in investigation of the stock structure of *E. tetradactylum* across Tropical Northern Australia (Newman et al., 2011). Others non marker that has been used in previous study is meristic and morphometric (Jaferian et al., 2010). All this method is influence by environmental condition and provides little information about reproductive pattern. Genetic marker is the best approach to study fish distribution and diversity between individual from same reproductive process because it more direct in defining fisheries stocks (Kenchington et al., 2003). Besides, genetic study also provide important insight into the amplitude and scale of dispersal and to identify the barrier that spatially limit the connectivity of marine population (Horne et al., 2011) that may be cause by physical barrier.

The difficulties to distinguish morphological character between *Eleutheronema* species lead to misidentification of species. The limitation in morphological identification and the decreasing of taxonomist expertise indicate the need for an accurate approach. The mitochondrial gene of the cytochrome c oxidase subunit I (COI) was found as a powerful tool in identifying animal taxa especially fish species. The study of population genetic structure of *Eleutheronema* fishes is able to provide information on population mixing and connectivity among location. The hypothesis for this study was that as an estuarine species with little tendency to migrate large geographical distances, *E. tetradactylum* and *E. rhadinum* may exist as several discrete populations along the Malaysian coastal water.

Therefore, this study is established to fulfill all the following objectives:

1. To characterize the genetic identity and distribution of *Eleutheronema* samples from Malaysian coastal water.
2. To examine the genetic variation and population genetic structure in *E. tetradactylum* along the Malaysian coastal waters.

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LIST OF PUBLICATIONS

Paper journal

Atikah, N. W., Esa, Y. B., Rozihan, M., Ismail, M. F. S., and Kamaruddin, I. S. (2020) Phylogenetic analysis revealed first report of *Eleutheronema rhadinum* lineage in the coastal waters of Malaysia. *Journal of Environmental Biology*, 41(5), 1424-1431.

Conference and Symposium

Atikah, N. W., Esa, Y. B., Rozihan, M., and Ismail, M. F. S. Population genetic structure and demographic analysis of *Eleutheronema rhadinum* (Jordan and Everman 1902) and *Eleutheronema tetradactylum* (Shaw 1804).TNCPI Universiti Putra Malaysia.13th to 15th November 2018.

Atikah, N. W., Esa, Y. B., Rozihan, M., and Ismail, M. F. S. Population genetic structure of *Eleutheronema tetradactylum* (Shaw 1804) in coastal waters of Malaysia inferred from microsatellites. ASEAN-FEN 9th International Fisheries Symposium, Seri Pacific Hotel, Kuala Lumpur. 18th to 21st November 2019.

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