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GENETIC STRUCTURE OF LONGTAIL SHAD [Tenualosa macrura (Bleeker, 1852)] POPULATIONS IN SARAWAK AND PHYLOGENETIC RELATIONSHIPS AMONG CLUPEIDS

NONA NABILAH BINTI AHMAD TARMIZI

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By

NONA NABILAH BINTI AHMAD TARMIZI

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May 2018

Chairman : Associate Professor Yuzine Bin Esa, PhD
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The tropical shad of genus *Tenualosa* (subfamily Dorosomatinae) is locally known in Malaysia as ikan terubok. They are important estuarine fishes, both commercially and culturally in many Asian countries including Malaysia. However, the phylogenetic relationship of family Clupeidae and genetic information on the population structure are still poorly understood. Therefore, this study examined the phylogenetic relationships of tropical shad of genus *tenualosa* and their relationships with other selected clupeids using sequence analysis of mitochondrial DNA cytochrome c oxidase I (COI) gene. This study also describe the genetic structure and level of genetic diversity of*Tenualosa macrura*samples, using sequence analysis of 604 base pair (bp) of the mitochondrial COI gene. A total of 51 *T. macrura*were analysed from 3 locations throughout Sarawak on Batang Sadong (*n* = 19), Samarahan (*n* = 21) and Mukah (*n* = 11). All samples were obtained from various localities throughout the coastal areas of Sarawak, Peninsular Malaysia and Bangladesh.The sequences were analysed using BLAST, BOLD, CLUSTAL X, MEGA 6.06, DnaSP programme, Network 5.003 and Arlequin software.

A total of 47 haplotypes were obtained from 132 sequences of clupeids. A total of 604bp of the COI sequences were obtained with 367bp conserved sites, 241bp variable sites and 227bp parsimony informative sites. The study proved the monophylies of the subfamiles Alosinae, Dorosomatinae and Clupeinae within the clupeids family. Between the two shads, namely, *Tenualosa macrura* and *Tenualosa toli*, high genetic divergences found between them (12.1-13.5 %) supported their taxonomic status as distinct species.

In total, 7 haplotypes of *Tenualosa macrura*were found, with 3singleton haplotypes and 4 haplotypes being shared among 3 populations. The experimental and statistic results of the mitochondrial COI analysis revealed that the haplotype diversity varied from 0.6257 to 0.6546 and nucleotide diversity ranged from 0.0012 to 0.0158, which pointed out that very high gene flow occur between the populations. Analysis of molecular variance (AMOVA) of Sadong, Mukah and Samarahan populations does not suggest existence of population structuring in *Tenulosa macrura* and suggested existence of a single population. The low level of mtDNA (2.36 %) differences currently found among *Tenualosa macrura* populations is probably due small number of samples used in the study.Overall, this study has managed to provide a better insight on the phylogeny, genetic identity and level of genetic differences and population structure of*Tenualosa macrura* and between fishes of Clupeidae.

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

POPULASI STRUKTUR GENETIK TERUBOK LAUT [*Tenualosa macrura* (Bleeker, 1852)] DI SARAWAK DAN HUBUNGAN FILOGENTIK ANTARA IKAN-IKAN CLUPEID

Oleh

NONA NABILAH BINTI AHMAD TARMIZI

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Pengerusi : Profesor Madya Yuzine Bin Esa, PhD
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Ikan Genus *Tenualosa* (subfamili Dorosomatinae) dikenali di Malaysia sebagai ikan terubok. Ikan-ikan ini amat pentingdaripada segikomersil dan juga sejarah latar belakangnya di Asia termasuk Malaysia. Walaubagaimanapun, hubungan filogenetik antara famili Clupeidae dan informasi genetik tentang struktur populasinya masih kurang dikaji. Oleh itu,kajian ini dijalankan untuk mengetahui hubugan filogenetik antara ikan terubok Genus*tenualosa* dan hubungkait antaraikan-ikan clupeid yang lain menggunakan analisis mitokondria DNA gen cytochrome c oxidase I (COI). Kajian ini juga menggambarkan struktur genetik dan tahap kepelbagaian genetik ikan terubok laut(*Tenualosa macrura*), menggunakan analisis urutan sepanjang 604 pasangan asas (bp) bagi gen COI mitokondria. Daripada analisis struktur populasi yang disimpulkan daripada urutan mitokondria COI, sejumlah 51 sampel*Tenualosa macrura* telah dianalisa dari 3 lokasi di seluruh Sarawak iaitu, Batang Sadong (*n* = 19), Samarahan (*n* = 21) and Mukah (*n* = 11). Keseluruhan sampel telah diperolehdi sepanjang kawasan pantai di Sarawak, Semenanjung Malaysia dan Bangladesh.Kesemua urutan dianalisis menggunakan perisianBLAST, BOLD, CLUSTAL X, MEGA 6.06, DnaSP programme, Network 5.003 dan Arlequin.

Sejumlah 47 haplotaip diperolehi daripada132 urutan ikan-ikan clupeid.Sepanjang 604bp daripada urutan COI telah diperoleh dengan 367bp ‘conserved sites’, 241bp ‘variable sites’ dan 227bp ‘parsimony informative sites’. Kajian ini juga membuktikan monophyli daripada subfamili Alosinae, Dorosomatinae dan Clupeinae dalam Famili Clupeidae. Antara dua jenis terubok iaitu*Tenualosa macrura* and *Tenualosa toli*, terdapat berbezaan genetik yang tinggi ditemui di antara mereka (12.1-13.5%) disokong oleh status taksonomi mereka sebagai spesies yang berbeza.

Secara keseluruhan, 7 haplotaip ditemui, dengan 3 haplotaip yang unik dan 4 haplotaip dikongsi antara tiga populasi itu. Keputusan eksperimen dan statistik analisis COI mitokondria mendedahkan kepelbagaian haplotaip iaitu nilai di antara 0.6257 hingga 0.6546 dan kepelbagaian nukleotida iaitu nilai di antara 0.0012 hingga 0.0158 yang membuktikan bahawa aliran gen sangat tinggi berlaku antara populasi-populasi itu. Analisis variasi molekul (AMOVA) daripada populasi Sadong, Mukah dan Samarahan tidak menunjukkan struktur populasi yang kukuh di antara *Tenualosa macrura* dan ini menujukkan kewujudan populasi tunggal di kawasan itu. Tahap perbezaan mtDNA yang rendah (2.36%) ditemui di antara populasi-populasi *Tenualosa macrura* mungkin adalah kerana bilangan sampel yang kecil digunakan di dalam kajian ini. Kesimpulannya, kajian ini telah berjaya memberikan gambaran yang lebih baik pada filogeni, identiti genetik, tahap perbezaan genetik dan struktur populasi bagi *Tenualosa macrura* dan di antara ikan-ikan Famili Clupeidae.

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This thesis was submitted to the Senate of the 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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LIST OF ABBREVIATIONS

°C	Degree Celcius
%	Percentage
®	Registered trademark
™	Trademark symbol
µl	Microliter
µM	Micromolar
л	Nucleotide diversity
mm	Millimeter
ml	Millilitre
bp	Base pair
kb	Kilobase
rpm	Revolutions per minute
dNTP	Deoxynucleotide triphosphate
tRNA	Transfer DNA
mRNA	Messenger RNA
nDNA	Nuclear DNA
COI	Cytochrome Oxidase I
mtDNA	Mitochondrial DNA
T. macrura	*Tenualosa macrura*
T. toli	*Tenualosa toli*
V	Voltage
UV	Ultra-violet

TL	Total length
SL	Standard length
ddH_2O	Double distilled water
d.f.	Degree of freedom
PCR	Polymerase Chain Reaction
et al	And others
NJ	Neighbour Joining
MP	Maximum Parsimony
ML	Maximum likelihood
MSN	Minimum spanning network
Kxy	Pairwise nucleotide difference
Dxy	Nucleotide substitution per site

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The family Clupeide is the fish under the order Clupeiformes which then subdivided into five subfamilies; Dussumieriinae, Clupeinae, Pellonulinae, Alosinae, and Dorosomatinae (Whitehead *et al*., 1988). Ccording to Agbayani (2004), the family Clupeidae currently has sixty six genera with 216 species. All of the species in this family lives from different ranges of habitat from freshwater to marine.

All fish from the family Clupeidae are called herring-like fish or commonly known asclupeids. According to Whitehead (1985), most of the clupeiforms are easily recognizable according to theirphysical characters of this following combination: forming abdominal scutes with a saw-like edge from modified belly scales, long and numerous gill rakers, absent of lateral line,body are short and moderately compressed, short anal fin andwell developed pelvic scutes.

There are currently five shads of the genus *Tenualosa* described worldwide; *Tenualosa ilisha*, *Tenualosa macrura*, *Tenualosa revesii*, *Tenualosa thibaudaui* and *Tenualosa toli*. However, only *T. macrura* (ocean Terubok) and *T. toli* (river Terubok) can be found in the Malaysian waters, both are existed along the coastal waters of Sarawak. *Tenualosa macrura* was previously widespread in South-east Asia but currently can only be found in the coastal waters of Sumatera and Borneo. They are important estuarine fishes, both commercially and culturally in many Asian countries including Malaysia (Blaber *et al*., 2003). Large numbers of people especially the local people, are dependent on the *Tenualosa* fishery in Sarawak in the form of artisanal and commercial fishery. Usually, the most commercially demand for both fishes, *T. toli* and *T. macrura* is for their eggs and this causes the catch landing of all *Tenualosa* has been depleted due to over-exploitation.These shads are unique because they are protandrus hermaphrodite (Blaber *et al*., 1996). The fisheries for both *Tenualosa* in Sarawak target the matured large females and it has been postulated that this protandry causes the species more vulnerable to overfishing and leads to their drastic declined in geographical area and population (Blaber *et al*., 2003).

1.2 Justification

The population genetic structure of the *T. macrura* has not yet been discovered. Therefore, it is necessary to provide a molecular analysis of mtDNA COI gene sequence to compare the genetic differentiation between *T. macrura* samples from several sites throughout the coastal areas of Sarawak. The studied data can provides baseline information for further preservation of *T. macrura*. Besides, the taxonomy of Clupeiformes (Whitehead, 1985) has extensively been studied but their phylogeny are still poorly resolved. Therefore, this study is designed to construct a molecular-based phylogeny for a number fish particularly from the Clupeidae family.

1.3 Research Objectives

The objectives of the study are as follows:

1. To determine the phylogenetic relationship of the Clupeidae family using sequences of the mitochondrial DNA cytochrome c oxidase I (COI) mtDNA gene; and
2. To examine the population genetic structure and level of genetic diversity among *Tenualosa macrura* samples.

1.4 Hypothesis

The hypothesis of this research are as follows:

1. The phylogenetic relationships among clupeids indicates the monophylies of the subfamilies Alosinae, Dorosomatinae and Clupeinae.
2. The study managed to describe the genetic population structure of *T. macrura* in Sarawak.

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