UNIVERSITI PUTRA MALAYSIA

PHYLOGENETIC RELATIONSHIPS BETWEEN Barbonymus gonionotus, Barbonymus schwanenfeldii, and Tor tambroides INFERRED FROM MITOCHONDRIAL CYTOCHROME C OXIDASE I (COI) SEQUENCE ANALYSIS

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This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

DEPARTMENT OF AQUACULTURE

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CERTIFICATION OF APPROVAL

DEPARTMENT OF AQUACULTURE

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This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

The study examined the phylogenetic relationships between the Cyprinids fishes *Barbonymus gonionotus*, *Barbonymus schwanenfeldii* and *Tor tambroides* using DNA sequencing of mitochondrial cytochrome c oxide I (COI) gene fragment using polymerase chain reaction. Sequencing of DNA samples were compared to the DNA reference sequence sample in GenBank using the BLAST program. Phylogenetic analysis of eight sequences using maximum likelihood (ML), minimum evolution (ME), and neighbor joining (NJ) resulted in similar tree topologies. The maximum pairwise genetic distance was *Barbonymus gonionotus* and *Tor tambroides* (15%), while the minimum value was between *Barbonymus gonionotus* and *Barbonymus schwanenfeldii* (11%). Comparative genetics of the genus Tor and Barbonymus not show a significant difference to the value of 16%.

ABSTRAK

Kajian ini mengkaji hubungan filogenetik antara tiga ikan siprinid *Barbonymus gonionotus, Barbonymus schwanenfeldii* dan *Tor tambroides* menggunakan penjujukan DNA mitokondria gen sitokrom c oksida I (COI) dengan menggunakan kaedah polymerase chain reaction. Jujukan DNA sampel dibanding dengan jujukan DNA sampel rujukan pada GenBank dengan mengunakan program BLAST. Analisis filogenetik lapan urutan menggunakan kaedah kebolehjadian maksimum (ML), evolusi minimum (ME), dan jiran menyertai (NJ) menghasilkan topologi pokok yang hampir sama. Jarak genetik maksimum adalah pasangan *Barbonymus gonionotus* dan *Tor tambroides* (15%) manakala nilai minimum adalah antara *Barbonymus gonionotus* dan *Barbonymus schwanenfeldii* (11%). Perbandingan genetik di antara genus Tor dan Barbonymus tidak menunjukkan perbezaan yang ketara dengan nilai 16%.

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%	percent
DNA	Deoxyribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
COI	C oxidase I
PFLP	Through Randomly Amplified Polymorphic
PCR	Polymerase chain reaction
UPM	University Putra Malaysia
µL	Microliter
ml	Milliliter
mg	Milligram
°C	Degree Celcius
Rpm	Rate per minute
g	Gram
TBE	Tris-borate-EDTA
MEGA	Molecular Evolutionary Genetic Analysis
dNTPs	Deoxynucleotide triphosphate
ddH_2O	Double distilled water
BLAST	Basic Local Alignment Search Tool
NJ	Neighbor-Joining
ME	Minimum Evolution
ML	Maximum Likelihood

CHAPTER 1

INTRODUCTION

In Malaysia, there are 4449 species of fish that have been identified according to Ministry of Natural Resources and Environment (2013) where 4000 species were found in marine water and 449 species were found in freshwater. Mainly for freshwater fishes, they are mostly found in rivers and streams, lakes, swamps and also reservoirs.

The largest and most abundant freshwater fishes in Malaysia come from family Cyprinidae in terms genera and species (Kamarudin and Esa, 2009). Malaysian cyprinids are very attractive in terms of colors and also play an important role as aquarium and ornamental fishes such as kelah and others. Lampam jawa or *B. gonionotus* is a well-known fish in Malaysia although it is not native in Malaysia.

Origin from Indonesia and is a dominant species especially in Java (Emmawati *et al*., 2013), this cyprinids are important in aquaculture sector as they used as a food sources besides becoming of economic part for the people around for the fish sell-buying activity. Besides that, it is also becomes a source of livelihood for a large rural

population (Khan *et al*., 1996). Currently, the freshwater fish catch has steadily decline due to degradation of aquatic habitats and also increasing levels of pollution besides over-exploitation. Thus, to enhance fisheries production as it to ensures the diversity genetic stock, migration and continuity of the species, conservation plays an important tool (Khan *et al*., 1996). Therefore, it is important to identify every species of fish, found in our waters so that every property land is not extinct due to the harm activities that carried out by our own hands.

The process of genetic identification and phylogenetic analysis comes with more benefits where sometimes scientists face difficulty in the identification of species identity. External information does not guarantee 100% difference identity of each fish species. This is a challenge to scientists to further investigate the identity of the identification process for each species of fish. One way to allow the scientists to obtain more accurate information is to use biotechnology, genetic methods or DNA based methods.

Identification of the identity can be done in biotechnology by using DNA barcoding. To match the sequence evidence item to a reference sequence through DNA sequence similarity searches or phylogenic reconstruction, it is the central concept for DNA barcoding (Dawnay *et al*., 2007). In a world of increasingly advanced and technological development has become increasingly fast and up-to-date, genetic

markers and DNA barcoding are one of the frequently used and increasingly important role in the identification, conservation and recognition of identity not only in fish, but also mammals, amphibians, reptiles and not to forget, humans. A global system from DNA-based barcode identification system also will provide a simple and universal tool that is applicable to all animal species, especially identification for fish species and products (Lakra *et al*., 2011). Recognition and identification of organisms such as in fish identity is not only subject to the identification process by looking at the external morphology alone, even by using DNA barcodes or genetic markers.

Besides that, genetic marker also can help to understand the history and evolution of population of the organism (Cavilli Sforza, 1998). Genetic marker is a way where genetic materials are used, especially DNA to tell about the species, populations or individuals and also allow us to characterize genetic diversity. Both animal and plants have their own DNA. The mitochondrial double-stranded DNA (mtDNA) molecule is small and containing several genes that characterize the mitochondrial genome and several regions corresponding to genes such as cytochrome c oxidase I (COI), cytochrome b, ND (gene coding for enzyme in NADH complex) and ribosomal and commonly used in population genetic, species identification assays and molecular phylogenetic analysis due to its high abundance in cell, high mutation rate and maternal inheritance (Catanese *et al*., 2009)

The variation in mitochondrial genome offers the possibility to using mtDNA as a marker in genetic studies of population and evolutionary. In practice, the mitochondrial variations can be studied in two ways which are RFLP and PCR-based. Through Randomly Amplified Polymorphic DNA (RFLP) technique, this method analysis the whole or a part of the genome through southern hybridization and for PCR-based, only amplification of a specific region and then followed by sequencing of PCR products (Malvee, 2008). mtDNA also evolves faster than nuclear DNA, implying the higher rate of mutation which is more likely to show variations at population or species level (Malvee, 2008). These make them suitable for population genetic and systematic analysis but there is only one main disadvantage, mtDNA is single locus, therefore variations only at one locus can be studied.

Therefore, this study was conducted to achieve the following objectives:

1. To examine the phylogenetic relationship between the three cyprinids: *Barbonymus gonionotus*, *Barbonymus schwanenfeldii* and *Tor tambroides*.
2. To evaluate the utility of cytochrome c oxidase I (COI) barcoding gene as genetic marker for species identification between the three species.

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