



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR PHYLOGENETICS OF PANGASIIDS IN PENINSULAR MALAYSIA  
USING MITOCHONDRIAL AND NUCLEAR GENES**

**AZLINA ZAINAB MATTAR**

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DERILMU BERBAKTI

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By

**AZLINA ZAINAB MATTAR**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
fulfillment of the Requirements for the Degree of Master of Science**

**May 2012**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**MOLECULAR PHYLOGENETICS OF PANGASIIDS IN PENINSULAR MALAYSIA USING MITOCHONDRIAL DNA AND NUCLEAR GENES**

By

**AZLINA ZAINAB BINTI MATTAR**

**May 2012**

**Chair: Siti Khalijah Daud, PhD**

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Pangasiidae is a large family of freshwater catfishes found throughout South and Southeast Asia. Typically, Pangasiidae are characterized by having smooth skin, two pairs of relatively short barbels and a subterminal mouth, a dorsal fin with a strong spine, small adipose fin, and a forked caudal fin which is separated from the anal fin. Some indigenous pangasiids, such as *Patin Buah* (*Pangasius nasutus*) and *Patin Muncung* (*Helicophagus waandersii*) are highly valuable and popular freshwater fish in Pahang and Perak. Unfortunately, currently, these fishes have become threatened species in rivers. Some introduced species of pangasiids from Thailand, such as *P. hypophthalmus*, has become one of the important freshwater fish for culture in Malaysia as a food source. Thus, it is vital to study the genetic relationships among various species of pangasiids in Malaysia. The aim of this study was to estimate phylogenetic relationships among species of the family Pangasiidae using both mitochondrial DNA (cytochrome c oxidase subunit 1 (COI), cytochrome b (Cyt *b*) and 16S rRNA (16S) and

nuclear genes (RAG1 and RAG2). The locus effectiveness in reveals phylogenetic relationships among species of the family Pangasiidae was also determined. A total of 42 samples of fish species belonging to family Pangasiidae were collected from Perak, Selangor and Sungai Pahang and fish farm. DNA from individual sample were extracted from muscle tissue and purified for the analysis. The methods used to infer the phylogeny of this family were neighbour joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses using MEGA4, PAUP and Mr. Bayes softwares. The Modeltest 3.7 was used to find the best model for ML and Bayesian analyses. The sequences were used and aligned together with an outgroup of *Ictalurus punctatus* sequences recovered from GeneBank. In this study, 462 bp, 903 bp, 543 bp, 949 bp and 877 bp were successfully sequenced from COI, Cyt *b*, 16S, RAG1 and RAG2 genes, respectively. The results from all the phylogenetic analyses showed similar patterns. The genetic distance values between pangasiid species based on COI, Cyt *b* and 16S ranged from 0.015 to 0.170 while the genetic distance values recovered from RAG1 and RAG2 ranged from 0.008 to 0.046. The trees of NJ, MP, Bayesian and ML based on mitochondrial DNA and nuclear genes produced similar topologies. The pangasiids were divided into two clades (Clade 1 and Clade 2), in which Clade 1 consisted of *P. nasutus*, *P. bocourti*, *P. micronemus* and *H. waandersii* while Clade 2 comprised only *P. hypophthalmus*. This showed, *H. waandersii* was found to be closer to *P. nasutus*, *P. bocourti*, *P. micronemus* than to *P. hypophthalmus*, eventhough it belongs to different genus. This showed that *P. nasutus*, *P. bocourti*, *P. micronemus* and *H. waandersii* were monophyletic with significant values of more than 0.97 for Bayesian probability. The results clearly showed that mitochondrial DNA and nuclear genes can be used to isolate the native species of Malaysian pangasiids, such as *P. nasutus*, *P.*

*micronemus*, *P. bocourti* and *H. waandersii* from the introduced species of *P. hypophthalmus*. Similar results revealed by both mitochondrial DNA and nuclear genes suggesting that these genetic markers are suitable to study the phylogenetic relationships among the pangasiid species, although *Cyt b* was found to be more effective than other markers. The results also suggest that the taxonomy of *H. waandersii* should be revised in the future. In order to validate genetic relationships among different species of pangasiids, additional indigenous species with larger sample size should be included in future studies.

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

**KAJIAN MOLEKUL FILOGENI PANGASIID DI SEMENANJUNG MALAYSIA  
MENGUNAKAN DNA MITOKONDRIA DAN GEN NUKLEAR**

Oleh

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Pangasiidae adalah famili ikan patin air tawar yang besar ditemui di seluruh Asia Selatan dan Asia Tenggara. Biasanya, Pangasiidae mempunyai ciri kulit licin, dua pasang sesungut yang agak pendek dan mulut jenis subterminal, satu sirip dorsal dengan spina yang kuat dan sirip adipos yang kecil dan sirip ekor bercabang yang terasing dari sirip dubur. Sesetengah pangasiid asli seperti Patin Buah (*Pangasius nasutus*) dan Patin Muncung (*Helicophagus waandersii*) adalah bernilai tinggi dan merupakan ikan air tawar yang popular di Pahang dan Perak. Malangnya, pada masa ini, ikan-ikan ini telah menjadi spesies terancam di sungai. Sesetengah spesies pangasiid yang diperkenalkan dari Thailand, seperti *P. hypophthalmus*, telah menjadi salah satu daripada ikan air tawar kultur yang penting di Malaysia sebagai sumber makanan. Oleh itu, kajian hubungan genetik di antara spesies-spesies pangasiid di Malaysia adalah penting. Objektif kajian ini adalah untuk melihat hubungan filogenetik antara spesies dalam famili Pangasiidae dengan menggunakan kedua-dua DNA mitokondria (sitokrom c oksida subunit 1 (COI), sitokrom b (*Cyt b*) dan subunit besar 16S rRNA (16S)) serta gen nuklear (RAG1 dan

RAG2). Keberkesanan lokus dalam mendedahkan hubungan filogenetik antara spesies dalam famili Pangasiidae juga telah ditentukan. Sejumlah 42 sampel spesies ikan dalam famili Pangasiidae ditangkap dari Sungai Pahang dan kolam ikan di Perak, Selangor dan Pahang. DNA daripada setiap sampel diekstrak dari otot tisu dan dituliskan untuk analisis berikutnya. Kaedah yang digunakan untuk membina filogeni bagi famili ini ialah analisis *neighbour joining* (NJ), *maximum parsimony* (MP), *maximum likelihood* (ML) dan *Bayesian* dengan menggunakan perisian MEGA4, PAUP dan Mr. Bayes. Perisian Modeltest 3.7 juga digunakan untuk menentukan model yang terbaik bagi analisis ML dan Bayesian. Jujukan ini telah digunakan dan dijajarkan bersama dengan jujukan *outgroup Ictalurus punctatus* dari GenBank. Dalam kajian ini, 462 bp, 903 bp, 543 bp, 949 bp dan 877 bp telah berjaya dijujukan dari COI, Cyt *b*, 16S, RAG1 and RAG2 gen, masing-masing. Keputusan daripada semua analisis filogenetik menunjukkan corak yang serupa. Nilai jarak genetik di antara spesies pangasiid berdasarkan COI, Cyt *b* dan 16S berjulat dari 0.015 kepada 0.170, manakala nilai jarak genetik yang diperolehi daripada RAG1 dan RAG2 berjulat dari 0.008 kepada 0.046. Pokok dari analisis NJ, MP, Bayesian and ML yang berasaskan DNA mitokondria dan gen nuklear menunjukkan topologi yang sama. Pangasiid spesies dibahagikan kepada dua klad (Klad 1 dan Klad 2), yang mana Klad 1 terdiri daripada *P. nasutus*, *P. bocourti*, *P. micronemus* dan *H. waandersii*, manakala Klad 2 terdiri daripada *P. hypophthalmus* sahaja. Keputusan menunjukkan *H. waandersii* lebih rapat kepada *P. nasutus*, *P. bocourti*, *P. micronemus* berbanding *P. hypophthalmus*, meskipun ia dari berlainan genus. Ini menunjukkan bahawa *P. nasutus*, *P. bocourti*, *P. micronemus* dan *H. waandersii* adalah monofiletik dengan nilai kebarangkalian Bayesian 0.97 yang signifikan. Hasil kajian ini menunjukkan penggunaan penanda DNA mitokondria dan



gen nuklear boleh mengasingkan spesies asal pangasiid di Malaysia, seperti *P. nasutus*, *P. miconemus*, *P. bocourti* dan *H. waandersii*, daripada spesies yang diperkenalkan iaitu *P. hypophthalmus*. Persamaan keputusan yang ditunjukkan oleh kedua-dua penanda genetik ini mencadangkan yang kedua-duanya adalah sesuai untuk kajian perhubungan filogenetik bagi spesies-spesies pangasiid, walau bagaimanapun telah dijumpai Cyt *b* adalah lebih berkesan dari penanda yang lain. Berdasarkan kepada keputusan kajian ini, taksonomi *H. waandersii* sepatutnya dikaji semula pada masa akan datang. Bagi mengesahkan perhubungan genetik antara spesies-spesies pangasiid yang berbeza, bilangan spesies asal dan saiz sampel perlu ditambah bagi kajian pada masa akan datang.

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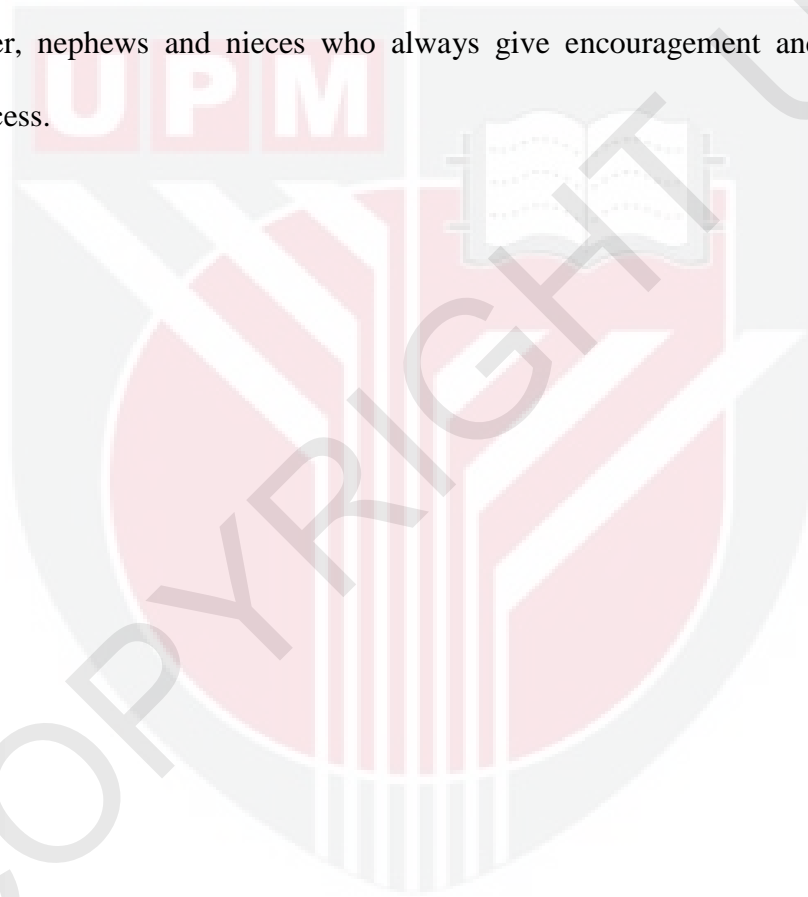
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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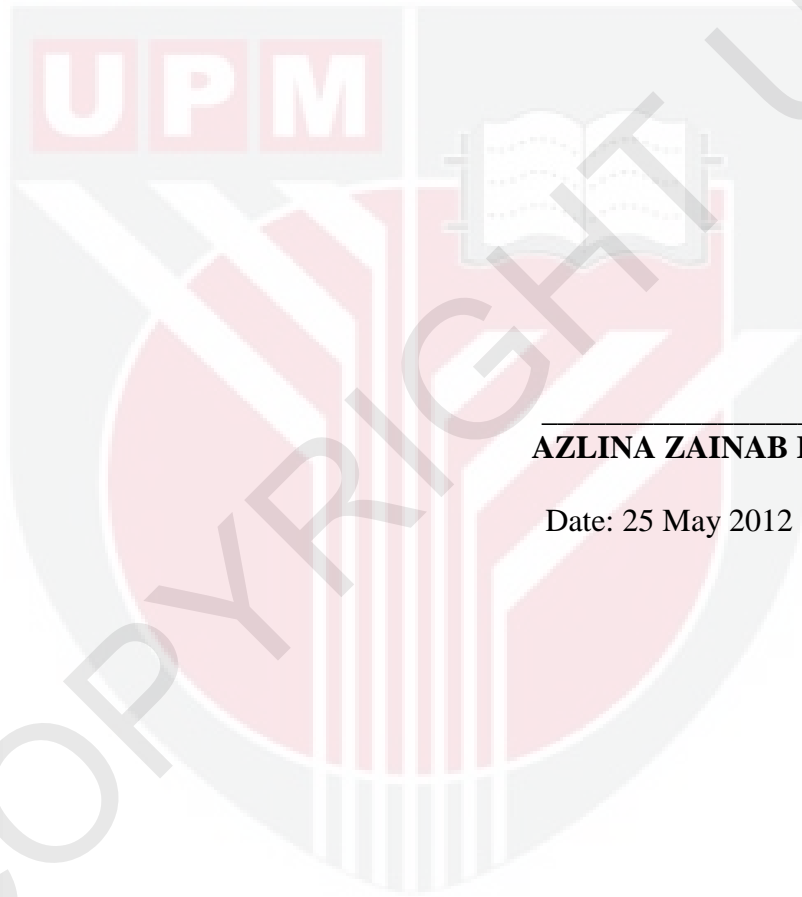
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Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.



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**AZLINA ZAINAB BINTI MATTAR**

Date: 25 May 2012

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