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GENETIC AND MORPHOMETRIC
VARIABILITY ASSESSMENT OF WILD
AND CAPTIVE POPULATIONS OF THE
CLIMBING PERCH, *Anabas testudineus*
TOWARDS DEVELOPMENT OF A
SELECTIVE BREEDING PROGRAMME

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Thesis Submitted in Fulfilment of the Requirement
for the Degree of Doctor of Philosophy in the
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LIST OF ABBREVIATIONS

Abbreviations

μL	Microlitter
AC	Alternate current
ANOVA	Analysis of variance
bp	Base pair
dNTP	Deoxyribonucleotide triphosphate
DOF	Department of Fisheries
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
GIFT	Genetically improved farm tilapia
ICLARM	International Center for Living Aquatic Resources Management
mM	Milimolar
MgCl₂	Magnesium Chloride
ng	Nanogram
ppt	Part per thousands
pmol	Picomol
PCR	Polymerase Chain Reaction
RAMs	Random amplified microsatellites
SGR	Specific growth rate
SPSS	Statistical Package for Social Science
rpm	Round per minutes
U	Unit

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Doctor of Philosophy

GENETIC AND MORPHOMETRIC VARIABILITY ASSESSMENT OF WILD AND CAPTIVE POPULATIONS OF THE CLIMBING PERCH, *Anabas testudineus* TOWARDS DEVELOPMENT OF A SELECTIVE BREEDING PROGRAMME

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March 2014

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In order to increase production of *Anabas testudineus*, selective breeding can be implemented to produce better performing stock. To initiate a successful selective breeding program, genetic variation is very important. This study was aimed to aid selective breeding of *Anabas testudineus* in Malaysia. Thus, morphological and molecular approach was adopted to investigate variation in hatchery stocks. In morphological approach, truss network method was applied to examine phenotypic variation of existing hatchery stocks. Cytochrome *b* gene was selected as a molecular marker to investigate the current status of genetic variation in hatchery stock. Growth performance of hatchery strain was conducted to evaluate performance of existing hatchery stock. Specific

microsatellite marker was isolated and developed for *Anabas testudineus*. Potential candidates for the base population of the selective breeding program were identified based on morphological and genetic variation of existing hatchery stocks. Morphological analysis of existing hatchery population in Malaysia revealed that each hatchery population belonged to distinct stock, where each population shows significant morphological variation between each stock. Based on cytochrome *b* analysis, all existing hatchery populations in Malaysia belonged to a single haplotype. In contrast, five distinct haplotypes was discovered based on genotypic data of wild *Anabas testudineus* population in Malaysia. Thus, it is recommended to introduce new genetic material from wild population to the synthetic base population to increase the genetic variation of the population. Based on growth performance, recommendation of possible candidate for the base population was put forth. Different level of growth performance was demonstrated by each population, candidate with the highest response to selection has been selected. Specific microsatellite markers for *Anabas testudineus* were isolated and developed to monitor progress of the selective breeding program and to mitigate the inbreeding and genetic loss. All of the information gathered is crucial to develop a sustainable selective breeding for *Anabas testudineus* and to improve quality and production of this species.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN VARIABILITI GENETIK DAN MORFOMETRIK KE ATAS
POPULASI LIAR DAN TERNAKAN IKAN PUYU, *Anabas testudineus* KE
ARAH PERKEMBANGAN SATU PROGRAM PEMBIAKAN PILIHAN**

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Dalam usaha untuk meningkatkan pengeluaran *Anabas testudineus*, pembiakan terpilih boleh dilaksanakan untuk menghasilkan baka yang lebih baik. Bagi memastikan keberkesanan sesuatu program pembiak bakaan terpilih, kepelbagaian variasi genetik adalah sangat penting. Kajian ini bertujuan untuk membantu pembiak bakaan *Anabas testudineus* di Malaysia. Oleh itu, pendekatan morfologi dan molekular telah diguna pakai bagi menilai kepelbagaian variasi di dalam baka yang sedia ada. Melalui pendekatan morfologi, teknik 'truss network' telah digunakan bagi mengkaji kepelbagaian variasi fenotip yang dimiliki oleh baka yang sedia ada. Bagi kaedah molekul, sitokrom b telah dipilih sebagai penanda molekul bagi menilai kepelbagaian

variasi dalam baka yang sedia ada. Manakala analisis 'growth performance' telah dijalankan bagi menilai prestasi tumbesaran 'strain' sedia ada. Penanda mikrosatelit khusus untuk *Anabas testudineus* telah diasingkan dan dibangunkan. Kajian ini juga telah mengenal pasti calon yang berpotensi bagi menghasilkan populasi asas program pembiak bakaan terpilih berdasarkan variasi morfologi dan genetik baka yang sedia ada. Analisis morfologi populasi baka sedia ada di Malaysia mendedahkan bahawa setiap populasi baka dari pusat penetasan adalah dari populasi yang berbeza, di mana setiap populasi menunjukkan perbezaan morfologi yang nyata antara baka yang sedia ada. Walau bagaimanapun, secara genetik kesemua populasi baka yang sedia ada di Malaysia datang dari haplotip yang sama. Namun, lima haplotip berbeza telah dikenal pasti dari *Anabas testudineus* liar di Malaysia. Oleh itu kajian ini telah mencadangkan bagi memperkenalkan bahan genetik baru dari populasi liar ke dalam populasi asas sintetik untuk program pembiak bakaan terpilih. Kajian ini juga telah mengenalpasti calon yang sesuai untuk populasi asas berdasarkan analisis prestasi tumbesaran. Dalam kajian ini berbagai prestasi tumbesaran telah diperhatikan, calon yang menunjukkan tindak balas paling tinggi terhadap pemilihan telah dipilih. Kajian ini juga telah mengasingkan dan membangunkan penanda mikrosatelit khusus untuk *Anabas testudineus* bagi tujuan pemantauan program pembiak bakaan terpilih dan mengurangkan 'inbreeding' dan kehilangan genetik dalam program pembiak bakaan terpilih.