MORPHOMETRIC AND PERFORMANCE OF Pangasianodon hypophthalmus (Sauvage, 1983) (♀) × Pangasius nasutus (Bleeker, 1976) (♂) HYBRID

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By

SITI FAIRUS MOHAMED YUSOFF

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DEDICATION

To my lovely mother, Bidah binti Wan Iberahim and my late father, Mohamed Yusoff bin Ismail (1949-2004). You are the best gift from ALLAH s.w.t. to me and thank you for being part of my journey in pursuing my dream and my reason to look forward to the next day.

Abstract of thesis presented to The Senate of Universiti Putra Malaysia in fulfilment of the requirement for The Degree of Doctor of Philosophy

MORPHOMETRIC AND PERFORMANCE OF *Pangasianodon hypophthalmus* (Sauvage, 1983) (♀) × *Pangasius nasutus* (Bleeker, 1976) (♂) HYBRID

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January 2019

Chairperson : Annie Christianus, PhD
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The present study was carried out to assess the morphometric variation and performance of crossbreed of *Pangasianodon hypophthalmus* (♀) and *Pangasius nasutus* (♂), (hybrid PH×PN) and its parental species; female, PH (*P. hypophthalmus* ♀ × *P. hypophthalmus* ♂); male, PN (*P. nasutus* ♀ × *P. nasutus* ♂). The first objective was employed to evaluate the feasibility (breeding performance, growth, survival) and biochemical composition (proximate, fatty acid, and amino acid profiles) of hybrid PH×PN and its parental species. Breeding, growth and survival performance of hybrid PH×PN based on fertilization, hatching, deformity, total length (TL), body weight (BW) and survival resulted in 73.50±1.34%, 65.43±2.24%, 9.93±1.70%, 32.12±5.56 mm, 107.71±9.91 mg, 62.5±6.50%, respectively, which were higher than in *P. nasutus*, 58.33±1.37%, 13.80±1.05%, 6.98±0.43%, 30.30±1.70 mm, 129.48±15.88 mg, 15.00±15.19%, respectively. Hybrid PH×PN showed higher protein content (25.09±0.22%) than both parental species (PH; 24.86±0.34%; PN: 23.55±0.24%) and higher lipid content (4.34±0.07%) than *P. hypophthalmus* (3.48±0.05%). Fatty acid and amino acid profiles varied between species, demonstrating high polyunsaturated fatty acids (PUFA) (26.32±1.66%) and total essential amino acids (EAA) (45.56±0.76%) in hybrid PH×PN than its parental species.

The second objective examined the morphological variation of hybrid PH×PN and its parental species. Results revealed a distinct vomerin and palatal teeth of hybrid PH×PN. Ten out of the thirty morphometric characters measured, which include prepelvic length, caudal peduncle length, dorsal fin length, pectoral fin length, adipose fin height, anal fin length, adipose fin length, interorbital length, distant snout to isthmus, and predorsal length are the strongest predictors that contributed meaningfully to the species discrimination. The third objective was carried out to characterize the genetic variation of hybrid PH×PN based on molecular marker using mitochondrial DNA (mtDNA) of Cytochrome Oxidase (I) (COI) gene to infer the maternal lineage of hybrid PH×PN and microsatellite marker applied to elucidate the genetic variation. Finding of COI gene revealed hybrid PH×PN shared the same haplotype (HPH1) as *P.*

hypophthalmus which proved the direction of maternal mating of hybrid PH×PN. Microsatellites marker showed genetic variability of hybrid PH×PN as observed over the loci. The fourth objective evaluated the resistance of hybrid PH×PN and its parental species towards bacteria, *Aeromonas hydrophila*. Results revealed the 96 h-LD_{50} value of hybrid PH×PN ($\times 10^{5.16}$ cfu mL^{-1}) was higher than *P. nasutus* ($\times 10^{3.51}$ cfu mL^{-1}) but lower than *P. hypophthalmus* ($\times 10^{6.67}$ cfu mL^{-1}). Immunological response of the respiratory burst and lysozyme activities, however, significantly higher in hybrid PH×PN prior to challenge but decreased subsequently at 46 and 96 h post challenged.

In conclusion, findings of this study revealed morphometric variation is beneficial in identifying and discriminating hybrid PH×PN from its parental species prior to using molecular markers. Genetic characterization using mitochondrial COI I inferred the maternal lineage of hybrid PH×PN. There is a promising performance of important traits in regard to growth, disease resistance, flesh quality particularly on the protein content than *P. nasutus*, revealing its potential as species for aquaculture.

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

MORFOMETRIK DAN PRESTASI *Pangasianodon hypophthalmus* (Sauvage, 1983) (♀) × *Pangasius nasutus* (Bleeker, 1976) (♂) HIBRID

Oleh

SITI FAIRUS MOHAMED YUSOFF

Januari 2019

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Kajian ini menilai variasi morfometrik dan prestasi hibrid yang dihasilkan daripada kacukan *Pangasianodon hypophthalmus* (♀) dan *Pangasius nasutus* (♂), ditanda sebagai hibrid PH×PN dan induk betina, PH (*P. hypophthalmus* ♀ × *P. hypophthalmus* ♂) dan induk jantan, PN (*P. nasutus* ♀ × *P. nasutus* ♂). Objektif pertama adalah untuk menilai kebolehupayaan (prestasi pembiakan, pembesaran, kemandirian hidup) dan komposisi biokimia (proksimat, profil asid lemak dan asid amino) hibrid PH×PN dan induknya. Prestasi pembiakan, tumbesaran dan kemandirian hibrid PH×PN berdasarkan persenyawaan, penetasan, kecacatan, panjang keseluruhan (TL), berat badan (BW) dan kemandirian adalah 73.50±1.34%, 65.43±2.24%, 9.93±1.70%, 32.12±5.56 mm, 107.71±9.91 mg, 62.5±6.50%, masing-masingnya, lebih tinggi berbanding *P. nasutus*, 58.33±1.37%, 13.80±1.05%, 6.98±0.43%, 30.30±1.70 mm, 129.48±15.88 mg, 15.00±15.19%, masing-masingnya. Hibrid PH×PN menunjukkan kandungan protein yang lebih tinggi (25.09±0.22%) daripada kedua-dua induk (PH; 24.86±0.34%; PN: 23.55±0.24%) dan lipid yang lebih tinggi (4.34±0.07%) daripada *P. hypophthalmus* (3.48±0.05%). Profil asid lemak dan asid amino berbeza setiap species dengan kandungan asid lemak tak tepu (26.32±1.66%) dan jumlah asid amino perlu (EAA) (45.56±0.76%) yang lebih tinggi daripada induknya.

Objektif kedua menilai variasi morfometrik bagi hibrid PH×PN dan induknya. Keputusan menunjukkan terdapat perbezaan vomerin dan gigi-palatal bagi hibrid PH×PN. Sepuluh daripada tiga puluh ciri morfometrik yang di ukur, iaitu panjang prepelvik, panjang kaudal pedunkel, panjang dorsal fin, panjang pectoral fin, ketinggian adipos fin, panjang anal fin, panjang adipos fin, panjang antara orbital, jarak snout ke isthmus dan panjang predorsal adalah ciri jangkaan yang kuat yang menyumbang kepada diskriminasi spesis. Objektif ketiga di jalankan untuk mencirikan variasi genetik bagi hibrid PH×PN berdasarkan penanda molekul mitokondria DNA (mtDNA) sitokrom oksidase (COI) (I) gen untuk menyimpulkan baka induk ibu dan penanda mikrosatelit untuk menjelaskan variasi genetik. Penemuan bagi COI gen mendedahkan hibrid PH×PN berkongsi haplotip yang sama (HPH1) dengan *P.*

hypophthalmus dan membuktikan baka bagi induk ibu. Penanda mikrosatelit meninjukkan kepelbagaian genetik hibrid PH×PN diperhatikan disepanjang lokus. Objektif keempat menilai ketahanan penyakit bagi hibrid PH×PN dan induknya terhadap bakteria, *A. hydrophila*. Keputusan menunjukkan nilai 96 h-LD_{50} bagi hibrid PH×PN ($\times 10^{5.16}$ cfu mL^{-1}) adalah lebih tinggi daripada *P. nasutus* ($\times 10^{3.51}$ cfu mL^{-1}) tetapi lebih rendah berbanding *P. hypophthalmus* ($\times 10^{6.67}$ cfu mL^{-1}). Tindakbalas imunologi bagi aktiviti letusan respirasi dan lisozim adalah lebih tinggi sebelum ujian cabaran tetapi berkurang kemudianya pada 46 and 96 h pasca ujian cabaran.

Kesimpulanya variasi morfometrik bermanfaat dalam mengenal pasti dan mengdiskriminasi hibrid PH×PN daripada induknya sebelum menggunakan penanda molekul. Perincian genetik menggunakan mitokondria COI menyimpulkan induk ibu hibrid PH×PN. Terdapat prestasi yang baik bagi baka baik merujuk kepada tumbesaran, daya tahan penyakit dan kandungan protein daripada *P. nasutus* menunjukkan potensinya sebagai spesis untuk akuakultur.

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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 Doctor of Philosophy. The members of the supervisory committee were as follows:

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- The research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

°C	Degree celsius
m	Meter
cm	Centimeter
mm	Millimeter
mg	Milligram
mM	Milimolar
mL	Mililiter
µL	Microliter
bp	Base pair
kb	Kilobase
rpm	Revolutions per minute
V	Volt
$MgCl_2$	Magnesium chloride
NCBI	National Center for Biotechnology Information
ML	Maximum Likelihood
MP	Maximum Parsimony
NJ	Neighbour Joining
Sp. Or spp	Species or species (plural)
cfu	Colony-forming unit
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dpi	dpi
EDTA	Ethylenediaminetetraacetic acid
H&E	Hematoxylin and eosin
HCl	Hydrochloric acid
LD_{50}	Median lethal dose
NaOH	Sodium hydroxide
NaCl	Sodium chloride
PCR	Polymerase chain reaction
TBE	Tris-borate-EDTA

TSA	Trypticase soy agar
UV	Ultra violet
h	Hour
EFA	Essential fatty acid
EAA	Essential amino acid
MUFA	Monounsaturated fatty acid

CHAPTER 1

GENERAL INTRODUCTION

1.1 Fisheries and Aquaculture Productions

The current world population of 7.3 billion is estimated to reach 9.7 billion by 2050 (United Nations, 2015). The growth is expected to increase the demand for global food production. Therefore, it is perceived as an imperative challenge due to economic uncertainty, competition for natural resources, and climate changes. To confront these challenges without a significant surge of the price, it is projected to increase approximately 70-80% of food production by 2050 (Godfray et al., 2010). Fish has a high prospect in contributing to global food production and nutrition security as it currently provides about 20% of all animal proteins (Godfray et al., 2010). It is highly possible to achieve this target which is through the intensification and sustainability of aquaculture productions.

Over the past decades, aquaculture appears to be the most rapid growth of food production industry, and it has exceeded the production of terrestrial livestock and dairy production with vast margin differences (Naylor, 2016). Aquaculture sector started to grow after the declining of the fisheries sector since the 1990s, with a production of only 90 million tons annually (FishStatJ, 2016). In 2014, about 70.8% of global fish production accounted for Asian countries, in which 88.9% comes from aquaculture and 56.5% from capture fisheries, with China dominates at 37.5%, while 9.3 % from South Asia, mainly India and Bangladesh as the top producers (FishStatJ, 2016). Meanwhile, ASEAN contributed 18.3% (30.6 million tons) of the total fish productions in the world (167.3 million tons) followed by Indonesia, Vietnam, and Myanmar. Presently, there are five main cultured species in the ASEAN regions, which are catfish (22%), tilapia (17%), shrimp (14%), carp (12%), and milkfish (9%). Catfish predominantly contributed to this production (Figure 1.1).

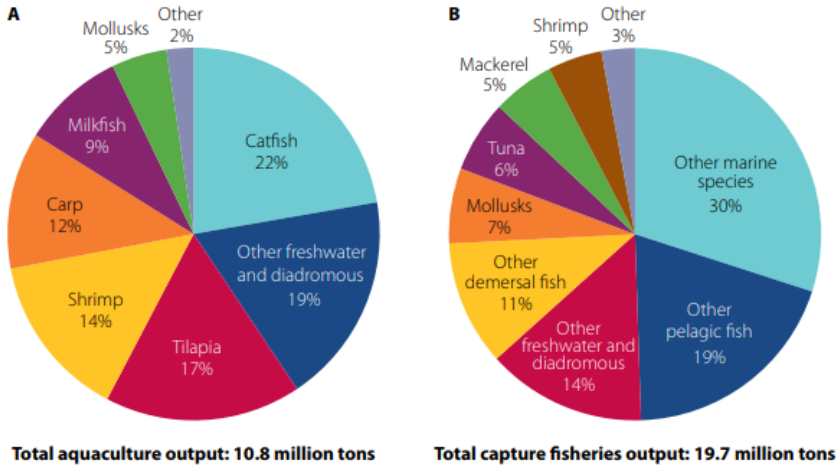

Figure 1.1: Share of major aquaculture fish species (A) and wild captured (B) of ASEAN's output in 2014
(Source: Chan et al., 2017)

Catfish from the family of Pangasidae, *Pangasianodon hypophthalmus* or locally known as 'Patin Siam' in Malaysia is the most commonly cultured species (John et al., 2015). It is well-known as one of the most promising species among the pangasiids family not only in Malaysia but other Southeast Asian countries (Phan et al., 2009; Bui et al., 2010; Zaman et al., 2017), it fetches a good price of MYR 10-15/kg (USD 2.5-3.7/kg) in local markets. It has high consumers acceptance due to its flesh quality with white flesh, low-fat content, and easily digestible protein, and most importantly without fishy odor, spines, and small bones (Orban et al., 2008). Another prominent species among the pangasiids species in Malaysia is *Pangasius nasutus* or locally known as 'Patin buah' flag for its outstanding organoleptic qualities and high retail price. In Selangor and Pahang, it can fetch a good price of RM 100-150/kg (USD 24.8-29.7/kg). Seedlings of this species are not produced by the local hatcheries (Department of Fisheries Malaysia, 2016) but collected from the wild.

1.2 Statement of Problems

Pangasius nasutus is considered as one of the popular freshwater food fishes and demands a good price in Malaysia. To date, farmers still rely on the wild catch to fulfill the market demands and this has led to declining of this species due to overfishing (Hassan, 2006). Even though the economic interest of this species has increased nowadays, mass production is still limited and published data which related to it is still scarce due to difficulty in egg collection. Improvement of female brood fish of *P. nasutus* for the egg collection purpose seems very hard due to most of the brood fish is caught from the wild and brood fish management prior induce breeding will take longer time. Nevertheless, easy sperm collection of *P. nasutus* male would be an advantage as it will give an opportunity to be crossbreed with other potential female pangasiids species, particularly species that having good traits.

To what extent a new hybrid has the potential to be commercialized as an aquaculture species is depending on its reproductive performance, viability to growth and a few other commercial traits. Basically, hybridization is implemented in genetic improvement program purposely to produce offspring that superior to their parental species either from the individual trait or overall traits that makes them economically profitable in aquaculture (Dunham & Masser, 2012). Hybridization is perceived as an alternative for genetic improvement due to an inexpensive method as compared to other methods (Bartley et al., 2001). Also, it is fast and an effective way to improve the important traits in the next generation through hybridization (Stuber, 1994; Birchler et al., 2010).

1.3 Research Justification

It is well-known hybrid fishes are produced in aquaculture aiming to improve its characteristics including growth, disease resistance, improve flesh quality, environmental tolerances, and various other traits to make the fish more profitable to be raised (Gjendrem, 2010). Even though there is difficulty in egg collection of *P. nasutus*, the justification selection of *P. nasutus* as a potential male (♂) in this study is primarily due to easy sperm collection and flesh quality preference. Meanwhile, *P. hypophthalmus* is chosen as a female (♀) due to easily available of female brood fish since this species is the common pangasiid species being bred and culture in Malaysia (John et al., 2015), high fecundity rate, and having good traits including fast growth, omnivorous species, highly tolerant to environmental conditions such as salinity, and dissolved oxygen, temperature or even pollution (Hill & Hill, 1994).

Breeding performance, survival, and growth are among the most important commercial traits to be evaluated in a new hybrid species targeted for mass productions, commercialization, and sustainability. Besides growth performance, nutritional quality of hybrid fishes produced are the important considerations to be evaluated. Flesh quality particularly biochemical compositions of amino acid and fatty acid contents are the important components in human diet. Therefore, when fish is suggested as a means of improving health through dietary intake, the nutrient contents of the hybrid is worth to be investigated. Study on morphological variation is is primarily for species identification. Morphometric variation was used in a first place prior to molecular work to discriminate the phenotype (Baharuddin et al., 2014), and rapid diagnostic is one of the key aspects and effective in identifying fish in stock management for the resources management, sustainability of aquaculture development, and managing domestication (Ibánez et al., 2017). At the molecular level, mitochondrial gene cytochrome C oxidase (COI) and microsatellites are among the accessible molecular markers used in hybrid studies to determine the genetic structure and identification of species (Teruo et al., 2011; Morgan et al., 2012; Gross et al., 2017). Mitochondrial DNA marker is known to reveal the maternal species due to maternal inheritance and used as the marker in identifying the maternal lineage of hybrids (Duong et al., 2017), and microsatellites marker is assisting in genetic analyses such as species identification and study on the population genetic structure (Jarne & Lagoda, 1996), particularly the polymorphic characteristic posses in microsatellites markers is important to reveal the genetic structure of hybrid PH×PN since the existence of genetic variation is crucial for the survival and fitness of the hybrid produced.

Hybridization appears to be an interesting approach to produce more resistant fish towards certain diseases as hybrid fishes could possibility demonstrated more resistant than its parental species. Since this hybrid is highly potential to be commercialized for mass production, assessing the morphometric variation for identifiction and its performance is crucial. Therefore, the research hypotheses and objectives were as follows:

1.4 Research Hypotheses

1) There is feasibility of crossbreed between *P. hypophthalmus* and *P. nasutus.*
2) There is dissimilarity of biochemical compositions, genetic variability, and disease resistant.
3) There is a morphometric variation of hybrid PH×PN and its parental species.

1.5 Objectives of Study

1) To asses the feasibility and biochemical compositions of hybrid PH×PN and its parental species.
2) To examine the morphometric variation of hybrid PH×PN and its parental species.
3) To characterize the genetic diversity of hybrid PH×PN and its parental species based on mitochondrial gene cytochrome C oxidase I (COI) and microsatellites marker.
4) To determine the disease resistance with regards to susceptibility towards *A. hydrophila* of hybrid PH×PN and its parental species.

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