MORPHOMETRICS OF SELECTED FOREST BABBLER SPECIES AND GENETIC VARIATIONS OF BLACK-CAPPED BABBLER [Pellorneum capistratum, (Temminck, 1823)] IN PENINSULAR MALAYSIA

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chair : Puan Chong Leong, PhD
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Babblers is one of the largest groups of insectivorous birds in Malaysian tropical rainforest with a high level of sympatry and morphological variations. Most babblers are short-distanced flyers and restricted to the interior forest, making them suitable candidate for biogeographic studies. Forest babblers are sensitive to forest disturbance due to their high habitat specificity and poor dispersal ability. Despite being common in lowland tropical forests, information on the morphological and genetic variations of the forest babblers in Malaysia are still lacking. Thus, this study was conducted to examine the morphometric variations of three forest babblers, namely the Black-capped Babbler (*Pellorneum capistratum*), Short-tailed Babbler (*P. malaccense*), and Chestnut-winged Babbler (*Cyanoderma erythropterum*) and further investigated the genetic variations of Black-capped Babbler populations from four sampling sites in Peninsular Malaysia. The main objective of this study was to elucidate the morphometric variations of these species in order to understand their ecological adaptations and functions in the forest habitats. The specific objectives were to develop a set of morphometric measurements using discriminant function analysis (DFA) which can be used for direct sex determination of these babbler species in the field. Genetic study of Black-capped Babbler was conducted to determine the genetic variations and connectivity of Black-capped Babbler populations in Peninsular Malaysia using double digest Restriction-site Associated DNA Sequencing (ddRAD-Seq). Eighty-six individuals comprising 22 Black-capped Babbler, 32 Short-tailed Babbler, and 32 Chestnut-winged Babbler were captured at Sungai Lalang Forest Reserves and Taman Botani Negara Shah Alam in Selangor, Panti Forest Reserves in Johor, and Bintang Hijau Forest Reserves in Perak. Based on seven morphometric traits taken from each species, results from DFA suggest that head and tail lengths can be used to distinguish the sexes of the Short-tailed Babbler with 73.3 % accuracy. For the Chestnut-winged Babbler, it was wing lengths and weight with 82.8 % accuracy. However, the DFA was not conducted for the Black-capped Babbler due to small sample size for females. Based on canonical function plots for Short-tailed and Chestnut-winged Babblers, there is no difference in morphometric measurements for all individuals at four sampling sites. However, for Black-capped Babbler, all individuals were separated morphologically based on sampling sites. Genetic analysis of 21 individuals of the Black-capped Babbler revealed high genetic connectivity among individuals based on the sampling sites. Based on Admixture analysis (k=1), all the 21 individuals were assigned into one putative population, indicating that most individuals were descended from closely related ancestor. However, based on morphometric data, it is shown that there is a small difference in morphology according to sampling sites. Netview analysis using k=4 showed that each individual in a population were clearly grouped bases on sampling sites. Overall, this study provides an easy and reliable method to facilitate direct sex determination for three babbler species in field, as well as revealing the population genetic structure of the Black-capped Babbler in Malaysia. Such information is crucial for the management and conservation of forest bird species in Malaysia and Southeast Asia.

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

MORFOMETRIK SPESIES BURUNG RIMBA HUTAN TERPILIH DAN VARIASI GENETIK BURUNG RIMBA TOPI HITAM [*Pellorneum capistratum* (Temminck, 1823)] DI SEMENANJUNG MALAYSIA

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Burung rimba ialah salah satu kelas burung pemangsa serangga yang terbesar di hutan hujan tropika di Malaysia dengan tahap simpatrik dan kepelbagaian morfologi yang tinggi. Kebanyakan burung rimba hanya dijumpai di kawasan pedalaman hutan, dan ini menyebabkan mereka menjadi calon yang sesuai bagi kajian biogeografi. Burung rimba hutan lebih sensitif kepada gangguan hutan disebabkan oleh keperluan ke atas habitat spesifik serta keupayaan penyebaran yang rendah. Walaupun mereka senang didapati di hutan hujan tropika tanah rendah, maklumat tentang variasi morfologi dan genetik burung rimba hutan masih kekurangan. Oleh itu, kajian ini dijalankan untuk meneliti variasi morfometrik tiga burung rimba hutan, iaitu Burung Rimba Topi Hitam (*Pellorneum capistratum*), Burung Rimba Ekor Putih (*P. malaccense*), dan Burung Rimba Merbah Sampah (*Cyanoderma erythropterum*) serta mengkaji variasi genetik populasi Burung Rimba Topi Hitam dari empat kawasan persampelan di Semenanjung Malaysia. Objektif utama kajian ini adalah untuk menentukan variasi morfometrik burung rimba hutan ini. Objektif spesifik adalah untuk menentukan satu set ukuran morfometrik menggunakan *Discriminant Function Analysis* (DFA) di mana ia boleh digunakan untuk mengenalpasti jantina burung rimba secara langsung di lapangan. Analisis genetik Burung Rimba Topi Hitam telah dijalankan untuk mengenalpasti corak variasi dan perhubungan genetik burung rimba hutan di Semenanjung Malaysia menggunakan *double digest Restriction-site Associated DNA Sequencing* (ddRAD-Seq). Lapan puluh enam individu merangkumi 22 Burung Rimba Topi Hitam, 31 Burung Rimba Ekor Putih, dan 31 Burung Rimba Merbah Sampah yang ditangkap di Hutan Simpan Sungai Lalang dan Taman Botani Negara Shah Alam di Selangor, Hutan Simpan Panti, di Johor, dan Hutan Simpan Bintang Hijau di Perak. Berdasarkan tujuh sifat morfometrik yang diambil daripada setiap spesies, hasil daripada DFA mencadangkan bahawa panjang kepala dan ekor boleh digunakan untuk membezakan jantina Burung Rimba Ekor Putih dengan ketepatan sebanyak 72.2 %. Untuk Burung Rimba Merbah Sampah, panjang sayap dan berat boleh digunakan dengan ketepatan sebanyak 82.8 %.

Walau bagaimanapun, DFA tidak dijalankan ke atas Burung Rimba Topi Hitam disebabkan oleh bilangan jantina betina yang kurang. Berdasarkan plot fungsi *canonical* bagi Burung Rimba Ekor Putih dan Merbah Sampah, tiada perbezaan dalam ukuran morformetrik bagi semua individu di empat kawasan persampelan. Tetapi, bagi Burung Rimba Topi Hitam, semua individu telah diasingkan mengikut ukuran berdasarkan kawasan persampelan. Analisis genetik untuk 21 individu Burung Rimba Topi Hitam menunjukkan perhubungan genetik yang tinggi di kalangan individu dalam setiap populasi. Berdasarkan analisis *Admixture* (k=1), kesemua 21 individu telah ditempatkan ke dalam satu populasi dan ini menunjukkan kesemua individu berasal daripada keturunan yang berdekatan. Tetapi, berdasarkan data morfometrik, ia menunjukkan perbezaan kecil dalam morfologi mengikut kawasan persampelan. Analisis *Netview* menggunakan k=4 menunjukkan kesemua individu di dalam populasi jelas dikumpulkan berdasarkan kawasan persampelan. Secara keseluruhan, kajian ini memberi satu kaedah yang senang dan boleh dipercayai untuk memudahkan proses mengenalpasti jantina untuk burung rimba di lapangan, serta menunjukkan populasi genetik Burung Rimba Topi Hitam di Malaysia. Maklumat ini sangat penting bagi pengurusan dan pemuliharaan burung hutan di Malaysia dan juga Asia Tenggara.

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I certify that a Thesis Examination Committee has met on 26th March 2019 to conduct the final examination of Norehan binti Abdl Jalil on her thesis entitled "Morphometric of Selected Babbler Species and Genetic Variations of Black-capped Babbler [*Pellorneum capistratum* (Temminck, 1823)] in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of 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

%	Percent
ºC	Degree Celsius
1X	One time
10X	Ten times
µl	Microliter
ml	Milliliter
mm	Millimeter
µg	Microgram
ng	Nanogram
mg	Milligram
cm	Centimeter
mM	Millimolar
min	Minute
s	Second
KCl	Potassium Chloride
KH_2PO_4	Monobasic Potassium Phosphate
NaCl	Sodium Chloride
rpm	Revolutions per minute
DNA	Deoxyribonucleic Acid
gDNA	Genomic DNA
cDNA	Complementary DNA
DFA	Discriminant Function Analysis
DF	Discriminant Function
SNP	Single Nucleotide Polymorphism
BEAST	Bayesian Evolutionary Analysis Sampling Trees
V	Volt

CHAPTER 1

INTRODUCTION

1.1 Babblers as Major Forest Birds

In most Asian tropical rainforests, babblers are the most diverse and well represented insectivorous bird group (Moyle et al., 2012; Powell et al., 2015). They are one of the major insectivorous bird groups in Malaysia (Yong et al., 2011; Mansor & Ramli, 2017) which often gather in mixed species flocks and display great ecological and morphological diversity (Delacour, 1946; Cibois, 2002). There are nearly 450 species of babblers widely distributed mainly in the Old-World regions (Collar & Robson, 2007). In Sundaland, there are approximately 275 babbler species comprising 50 genera (Sheldon et al., 2015), with 39 resident species found in Peninsular Malaysia (Robson, 2008).

Generally, most babbler species are short-distanced flyers with relatively short and rounded wings (Jeyarajasingam, 2012). Thus, there are highly territorial, which makes them a suitable candidate for biogeographic studies (Moyle et al., 2012). Although most of them are sedentary and elusive, their presence can be detected through vocalisation. In the tropics, babblers have been used to investigate the effects of forest disturbance (Hamer et al., 2015) and indeed some species have been shown to be sensitive to forest fragmentation (Moradi and Mohamed, 2010; Yong et al., 2011).

Previous studies on babblers have mainly focused on phylogenetics (i.e. Cibois et al., 2003; Gelang et al., 2009; Moyle et al., 2012) as well as foraging strategies (i.e. Mansor et al., 2015; Styring et al., 2016; Mansor & Ramli, 2017). However, knowledge on morphometric and genetic variations among babbler species in Malaysia remains poorly understood. Examining their morphological characteristics are useful in answering many ecological questions pertaining to adaptation, survival as well as sexual dimorphism.

1.2 Problem Statements and Research Significance

In this study, I examined the morphometric variations of three forest babbler species, namely the Black-capped Babbler (*Pellorneum capistratum*), Short-tailed Babbler (*P. malaccense*) and Chestnut-winged Babbler (*Cyanoderma erythropterum*) and further investigated the genetic variations of four Black-capped Babbler populations in Peninsular Malaysia. Previous studies have shown that most babblers have high habitat and foraging specificity (Yong et al., 2011; Mansor & Ramli, 2017), and are particularly sensitive to forest fragmentation (Moradi & Mohamed, 2010; Yong et al., 2011).

For example, terrestrial species such as Black-capped and Short-tailed Babblers favour microhabitats that are humid with high leaf litter cover and dense canopy (Moradi et al., 2008) in which they forage for insects on the forest floor. Such habitats allow them to perform their foraging tactics, i.e. gleaning for insects on the lower forest stratum (Mansor & Ramli, 2017). Hence, as ground foragers, they are more vulnerable to predators as compared to arboreal foragers and have been shown to decline in numbers due to disturbance (Hamer et al., 2015).

On the other hand, arboreal species such as the Chestnut-winged Babbler is more social and often spend time either perching or moving through trees in mixed species flocks. They can also be found in habitats along the forest edge (Moradi & Mohamed, 2010) and forage at the understory level in the forests (Styring et al., 2016). Unlike the ground foragers, arboreal species may have relatively lower predation risks by joining mixed species flocks (Mansor & Ramli, 2017).

Forest birds play a very important role in the terrestrial forest ecosystem (Stratford & Sekercioglu, 2015). While most frugivores are agents for seed dispersal and pollination, insectivores, on the other hand, control the insect populations in the forest (Sekercioglu, 2006). Although there is a lack of information on the ecological functions of forest babblers, several studies have proven that the presence of insectivorous birds can help to reduce agricultural plant damage such as cocoa (Van Bael et al., 2008) coffee (Johnson et al., 2010), and pigeon pea crop (Bharucha & Padate, 2010).

This study focused on the three resident babbler species that are either terrestrial or arboreal. Terrestrial babbler is a suitable candidate for biogeographic studies due to their solitary and highly territorial behaviour (Moyle et al., 2012). On the other hand, arboreal babbler is more gregarious and is able to move around in the understory level of the forest. Thus, examining their morphological variations will aid in better understanding of their ecological adaptations and functions in the forest habitats.

Niche segregation involving various foraging tactics has been demonstrated in Malaysian babblers (Mansor & Mohd Sah, 2012; Mansor et al., 2015; Styring et al., 2016; Mansor & Ramli, 2017) and this allows them to coexist in the same habitat. In relation, intersexual resource partitioning is another aspect that is worth to be investigated. This is because, sexual dimorphism is often related to sexual selection, mating systems, as well as resource partitioning, which aid in ecological knowledge and conservation of a species.

Nevertheless, most babbler species are similar in terms of physical appearance between males and females and size dimorphisms, if any, may be subtle or overlapped (Wells, 2010). Thus, it is hard to determine their sex solely based on visual inspection. One of the common methods in avian sex determination is molecular sexing using Chromo-Helicase DNA binding (*CHD*) gene (Lee et al., 2010). However, this method can only be done in a laboratory setting. Thus, there is a need to provide a simple, rapid, and less expensive method to determine the sex of wild birds such as babblers in the field by using morphometric analysis.

Analysing their morphological traits and genetic variability present a way to predict their survival in the forest ecosystem. This study was conducted to examine whether differentiation in morphology can be used for species identification between the babbler species and whether morphological measurements can be used to distinguish males from the females. The knowledge on sex ratio of wild birds is crucial in population, behavioural, as well as evolutionary history of a species, aiding in understanding and thus conservation of a species.

On the other hand, genetic study which focused on the Black-capped Babbler was conducted to examine the level of genetic variations of this solitary species in the forests of Peninsular Malaysia. Up until now, genetic information pertaining to the Black-capped Babbler from Peninsular Malaysia (*P. c. nigrocapitatum*) is very limited and inadequate (Cai et al., 2018) as compared to Short-tailed and Chestnut-winged Babblers which are relatively well studied both ecologically and genetically (Sadanandan & Rheindt, 2015; Mansor et al., 2015; Lim et al., 2017; Yong et al., 2009).

The Black-capped Babbler was chosen as a focal species for genetic study because it is a foliage gleaning species with highly territorial behaviour. It is often solitary and does not forage with other species. In addition, as a terrestrial species, they have poor dispersal ability as they do not travel too far (Myers, 2009). This attribute makes their distribution restricted in the forest interior and relatively smaller home range (Mohamed et al., 2013). Small bird populations which have been isolated over a long period of time could suffer from inbreeding depression as individuals from such populations would mate with their close relatives (Charlesworth & Willis, 2009).

Therefore, the work described in the following sections attempt to provide an overview on genetic aspects on this species which will be useful for the management and conservation of the species. The understanding of the genetic variations and connectivity of forest-dwelling species such as Black-capped Babbler is important to ensure their survival in increasingly fragmented habitat. The knowledge of population structure provides information on dispersal ability and gene flow of the species, which is critical to understand the species ability to survive in fragmented habitat (Brown et al., 2004).

1.3 Research Objectives

The main objective of the study was to examine the morphometric variations of three forest babbler species, namely Black-capped, Short-tailed and Chestnut-winged Babblers and to study genetic variations of Black-capped Babblers in Peninsular Malaysia. The specific objectives were:

1) To develop a set of morphometric measurements that can be used for direct sex determination of these three babbler species,

2) To examine interspecific morphological variations among these three babbler species from four different forests across Peninsular Malaysia, and

3) To determine the pattern of genetic variation and connectivity of one of the focal species, i.e. Black-capped Babbler populations across Peninsular Malaysia using double digest Restriction-site Associated DNA Sequencing (ddRAD-Seq).

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