



UNIVERSITI PUTRA MALAYSIA

***MORPHOMETRY OF AND GENETIC VARIATION IN MTDNA OF THE
EDIBLE NEST SWIFTLET (*Aerodramus fuciphagus* Thunberg)
IN MALAYSIA***

KOLADE OLUWASEYI MODUPE

FP 2014 25



**MORPHOMETRY OF AND GENETIC VARIATION IN MTDNA OF THE
EDIBLE NEST SWIFTLET (*Aerodramus fuciphagus* Thunberg)
IN MALAYSIA**

By

KOLADE OLUWASEYI MODUPE

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

July 2014

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



Abstract of thesis to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Master of Science

MORPHOMETRY OF AND GENETIC VARIATION IN MTDNA OF THE EDIBLE NEST SWIFTLET (*AERODRAMUS FUCIPHAGUS* THUNBERG) IN MALAYSIA

By

KOLADE OLUWASEYI MODUPE

July 2014

Chairman : Professor Jothi Malar Panandam, PhD
Faculty : Agriculture

A. fuciphagus (edible nest swiftlets) naturally inhabit the limestone caves of South East Asia, but recently swiftlet ranching in man-made houses has become popular. These swiftlets are of commercial importance because of their unique nests produced wholly of the mucilaginous secretion from the salivary glands and highly valued for their nutritional, aphrodisiac and medicinal properties. Limited works are available on the genetics of this swiftlet, especially in Malaysia. There is also a lack of knowledge about the spatial delineation of the swiftlet species in Malaysia.

This study was carried out to elucidate information on genetic variability of *A. fuciphagus* by analysing seven mtDNA genes (*cytb*, *ND2*, *ND3*, *ND5*, *12S rRNA*, *16S rRNA* and *COI*), with the hope of identifying DNA barcodes for the purpose of traceability of the edible bird nests (EBNs). Morphometric parameters of the birds were also evaluated so that the variations observed among locations may be associated with variations among the molecular markers.

For the morphometric study, 33 birds captured humanely from five locations in West Malaysia (Bachok, Kota Bharu and Tanah Merah in Kelantan, Kuala Terengganu in Terengganu, and Sungkai in Perak) were measured for beak thickness, length of beak, tail length, leg length and wing length. There were no significant differences in the beak thickness of the EBN swiftlets from the five locations, while significant ($P < 0.05$) differences were observed for the other parameters among some locations. There were no significant correlation between body weight and beak thickness, beak length, leg length, wing length and tail length there were no association existing between the body weight and morphometric parameters. Generally, there seemed to be no clear pattern of geographical variations for the morphometric traits investigated among the locations.

For the mtDNA analysis, DNA samples, extracted from blood, tissue or nests, of 28 EBN swiftlets from six locations (Sungkai in Perak, Tanah Merah in Kelantan, Kuala Terengganu in Terengganu, Kota Tinggi in Johor, Gua Madai and Pitas in Sabah) were used. The DNA was subjected to PCR amplification of seven mtDNA gene loci, namely *cytb*, *ND2*, *ND3*, *ND5*, *12S rRNA*, *16S rRNA* and *COI*. Sequencing of the amplicon revealed a total of 33 haplotypes across the loci. The *cytb* and *ND2* loci had the highest variation with each exhibiting eight haplotypes. The *12S rRNA*, *16S rRNA*, *COI* and *ND5* loci had five, six, four and five haplotypes, respectively; *ND3* had only one. The Tajima's *D* showed a negative value for all the loci ($P > 0.05$), while the Fu and Li's *D* values were negatives for all the loci except *16S rRNA*. Estimates of genetic differentiation for the mtDNA loci did not show any genetic structure within or between the locations. The median joining network (MJN) tree for all the gene loci showed that the EBN swiftlet populations could not be distinguished geographically. The shared haplotypes suggest high gene flow between locations. The mismatch distributions for all loci were multimodal in shape except for the *ND5* locus. The mtDNA loci indicated lack of spatial delineation and the unsuitability of these markers for DNA barcoding. However, the results showed that the overall genetic diversity in the species was relatively high indicating a genetically healthy population. The data obtained from this study will be useful to monitor the effects of swiftlet ranching on the *A. fuciphagus* population and in the management of the genetics of this species.

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

MORFOMETRI DAN VARIASI GENETIK DALAM MTDNA BURUNG WALIT (*AERODRAMUS FUCIPHAGUS* THUNBERG) DI MALAYSIA

Oleh

KOLADE OLUWASEYI MODUPE

Julai, 2014

Pengerusi : Professor Jothi Malar Panandam, PhD

Fakulti: Pertanian

A. fuciphagus (burung walit) secara semula jadi mendiami gua batu kapur di Asia Tenggara, tetapi baru-baru ini penternakan burung walit dalam rumah buatan manusia telah menjadi popular. Burung walit mempunyai kepentingan komersial kerana sarang unik mereka dihasilkan penuh daripada rembesan *mucilaginous* dari kelenjar air liur dan sangat bernilai untuk ciri-ciri pemakanan, afrodisiak dan perubatan. Kerja mengenai genetik burung walit adalah terhad, terutama di Malaysia. Terdapat juga kekurangan pengetahuan mengenai *spatial delineation* spesies burung walit di Malaysia.

Kajian ini telah dijalankan untuk menjelaskan kepelbagaian genetik *A. fuciphagus* dengan menganalisis tujuh gen mtDNA (*cytb*, *ND2*, *ND3*, *ND5*, *12S rRNA*, *16S rRNA* dan *COI*), dengan harapan untuk mengenal pasti barcode DNA untuk tujuan kebolehsasaran sarang burung yang boleh dimakan (EBN). Parameter morfometrik burung tersebut juga dinilai supaya variasi yang diperhatikan di antara lokasi boleh dikaitkan dengan variasi antara penanda molekul.

Untuk kajian morfometrik, 33 burung yang ditangkap berperikemanusiaan dari lima lokasi di Malaysia Barat (Bachok, Kota Bharu dan Tanah Merah di Kelantan, Kuala Terengganu di Terengganu, dan Sungkai di Perak) diukur untuk ketebalan paruh, panjang paruh, panjang ekor, kaki panjang dan panjang sayap. Tidak ada perbezaan yang signifikan dalam ketebalan paruh burung walit dari lima lokasi, manakala perbezaan yang ketara ($P < 0.05$) antara parameter lain diperhatikan di kalangan sesetengah lokasi. Terdapat korelasi yang tidak ketara antara berat badan dan panjang paruh, panjang ekor dan kaki panjang. Secara amnya, terdapat seolah-olahnya tiada corak variasi geografi bagi sifat-sifat morfometrik yang disiasat antara lokasi.

Untuk analisis mtDNA, sampel DNA, yang diekstrak daripada darah, tisu atau sarang, sebanyak 28 burung walit dari enam lokasi (Sungkai di Perak, Tanah Merah di Kelantan, Kuala Terengganu di Terengganu, Kota Tinggi di Johor, Gua Madai dan Pitas di Sabah) telah digunakan. DNA tersebut telah tertakluk kepada amplifikasi PCR tujuh lokus gen mtDNA, iaitu *cytb*, *ND2*, *ND3*, *ND5*, *12S*, *16S* dan *COI*. Urutan amplicon mendedahkan sejumlah 33 haplotip seluruh lokus. Lokus *cytb* dan *ND2* mempunyai variasi yang tertinggi dengan tiap satu mempamerkan lapan haplotip. Lokus *12S rRNA*, *16S rRNA*, *COI* dan *ND5* mempunyai lima, enam, empat dan lima haplotip, masing-masing; *ND3* mempunyai hanya satu. D Tajima menunjukkan nilai negatif untuk semua lokus ($P > 0.05$), manakala nilai D Fu dan Li adalah negatif untuk semua lokus kecuali *16S rRNA*. Anggaran pembezaan genetik untuk lokus mtDNA tidak menunjukkan sebarang struktur genetik dalam atau di antara lokasi. Median joining network (MJN) tree untuk semua lokus gen menunjukkan bahawa populasi burung walit tidak boleh dibezakan dari segi geografi. Haplotip yang dikongsi mencadangkan aliran gen tinggi antara lokasi. Taburan ketidaksepadanan untuk semua lokus adalah berbentuk *multimodal* kecuali bagi lokus *ND5*. Lokus MtDNA menunjukkan kekurangan *spatial delineation* dan ketidaksesuaian penanda ini untuk barcode DNA. Walau bagaimanapun, keputusan menunjukkan bahawa kepelbagaian genetik keseluruhan dalam spesis tersebut agak tinggi menunjukkan populasi yang sihat dari segi genetik. Data yang diperolehi daripada kajian ini akan berguna untuk memantau kesan penternakan burung walit ke atas populasi *A. fuciphagus* di Malaysia dan dalam pengurusan genetik spesis ini.

ACKNOWLEDGEMENTS

First and foremost, my honest appreciation goes to the all mighty God for giving me the grace and strength to complete this study.

I am most grateful to my supervisor Professor Dr. Jothi Malar Panandam, for her patience, tireless support, willingness to help and encouragement, kindness and guidance throughout the research and during the preparation of the thesis. I am very much indebted to the other members of my supervisory committee, namely Professor Dr. Tan Soon Guan and Dr Sumita Sugnaseelan, for their encouragement, constructive discussion, excellent advice and suggestions throughout the project.

I wish to express my sincere gratitude to everyone who helped me or contributed to the success of my work; special thanks to the staff of the Genetic Laboratory, Miss Kamariah Jamhari, for her assistance. I would like to appreciate Dr Reuben for allowing me to use his laboratory facilities. I would also like to thank staff members of the Parasitology Laboratory, Faculty of Veterinary Medicine for your support and encouragement during the course of my study.

My sincere appreciation to Dr Saeid Nikbin, Dr Haytham hago Abdelwahid, Dr Gimba Ido, Dr Ope Onilude, Mr Yow Weng Kit, Dr Ong and Miss Maisarah for the invaluable assistance rendered at one point in time or other.

Finally, I would like to thank the 'The Lords Chapel'; you are truly a wonderful family to be associated with. I would also like to appreciate my fiancé, Adebayo Adewale for his moral support and care, and my family members for their prayers. Thank you for always being there in my joy, pains and trying times.

I certify that a Thesis Examination Committee has met on 7 July 2014 to conduct the final examination of Kolade Oluwaseyi Modupe on her thesis entitled "Morphometry of and Genetic Variation in MTDNA of The Edible Nest Swiftlet (*Aerodramus fuciphagus* Thunberg) in 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.

Members of the Thesis Examination Committee were as follows:

Halimatun binti Yaakub, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Reuben Sunil Kumar Sharma, PhD

Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Lau Wei Hong, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Badrul Munir Md Zain, PhD

Associate Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)



NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 23 October 2014

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee are as follows:

Jothi Malar Panandam, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Sumita Sugnaseelan, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Tan Soon Guan, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No: Kolade Oluwaseyi Modupe/ GS32472

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee _____

Signature: _____
Name of
Member of
Supervisory
Committee _____

Signature: _____
Name of
Chairman of
Supervisory
Committee _____



TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATION	xvi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 <i>Aerodramus fuciphagus</i>	3
2.1.1 Classification and Taxonomy	3
2.1.2 Morphology	3
2.1.3 Echolocation	4
2.1.4 Breeding	4
2.1.5 Foraging Habit	6
2.1.6 Conservation Status	6
2.2 Morphometric Characteristics	7
2.3 Genetic Variation	9
2.4 Molecular Markers	9
2.5 Mitochondria DNA Marker	11
2.6 Barcoding	13
3 MORPHOMETRIC VARIABILITY OF <i>AERODRAMUS FUCIPHAGUS</i>	14
3.1 Introduction	14
3.2 Materials and Method	15
3.2.1 Sample Collection	15
3.2.2 Morphometric Parameter Measurement	16
3.2.3 Data Analysis	17
3.3 Result	19
3.4 Discussion	28
4 MITOCHONDRIA DNA VARIABILITY IN <i>AERODRAMUS FUCIPHAGUS</i>	30
4.1 Introduction	30

4.2	Material and Method	30
4.2.1	Location and Samples	30
4.2.2	DNA Extraction Techniques	31
4.2.3	Polymerase Chain Reaction (PCR) Amplification	33
4.2.4	PCR Amplicon Purification	36
4.2.5	Cloning	36
4.2.6	Plasmid Extraction	37
4.2.7	Sequencing of Purified Plasmid	37
4.2.8	Analysis of MtDNA data	38
4.3	Result	40
4.3.1	Genetic Variability of MtDNA loci	40
4.3.2	Genetic Diversity of the MtDNA Gene Loci	85
4.3.3	Multilocus analysis of the six populations.	87
4.4	Discussion	89
4.4.1	Variation within Individual Markers	89
4.4.2	Variation within Location	90
4.4.3	Variation Among Locations	91
4.4.4	Barcoding of EBN Swiftlet	92
5	GENERAL DISCUSSION AND CONCLUSION	93
	REFERENCES	95
	APPENDICES	110
	BIODATA OF STUDENT	116
	LIST OF PUBLICATION	117

LIST OF TABLES

Table	Page	
3.1	Sampling locations and sample sizes for EBN swiftlet	16
3.2	Analysis of variance for morphometric parameters of the EBN swiftlets from five locations	20
3.3	Means of morphometric parameters of the EBN swiftlets from five locations	21
3.4	Correlation coefficient of morphometric parameters of the EBN swiftlets from five locations	23
3.5	Eigen values and percentage of total variance along with the rotated component matrix	23
3.6	Bivariate correlation between weight and morphometric parameters	23
3.7	Morphometric parameters corrected with weight per location	24
4.1	Sampling locations, sampling type and sampling sizes for EBN swiftlet	31
4.2	PCR protocol conditions showing the number of cycles, and annealing, denaturisation and extension temperatures for all the loci used	34
4.3	MtDNA loci selected for cross amplification of <i>A. fuciphagus</i> DNA and information on primers used	35
4.4	Haplotypes showing the polymorphic sites, base polymorphism and distribution across locations for MtDNA <i>cytb</i> region	41
4.5	Genetic diversity indices of the MtDNA <i>cytb</i> region in six populations across Malaysia	42
4.6	Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in MtDNA <i>ND2</i> region	49
4.7	Genetic diversity indices for the MtDNA <i>ND2</i> region in six populations across Malaysia	50
4.8	Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in MtDNA <i>ND5</i> region	58
4.9	Genetic diversity indices for the MtDNA <i>ND5</i> region in six populations across Malaysia	59
4.10	Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in MtDNA <i>16S rRNA</i>	66
4.11	Genetic diversity indices for the MtDNA <i>16S rRNA</i> region in six populations across Malaysia	67

4.12	Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in MtDNA <i>12S rRNA</i> region	73
4.13	Genetic diversity indices for the MtDNA <i>12S rRNA</i> region in six populations across Malaysia	74
4.14	Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in MtDNA <i>COI</i> region	80
4.15	Genetic diversity indices for the MtDNA <i>COI</i> region in six populations across Malaysia	81
4.16	Genetic divergence of all the gene loci across all populations and the model used for each locus	86
4.17	Genetic identity and distance of all the gene loci across all populations	87



LIST OF FIGURES

Figure	Page	
3.1	Map of Malaysia showing the different EBN swiftlet sampling sites	15
3.2	Illustration showing the morphometric traits measured.	17
3.3	Scatter plot of body weight and beak thickness	25
3.4	Scatter plot of body weight and beak length	25
3.5	Scatter plot of body weight and leg length	26
3.6	Scatter plot of body weight and tail length	26
3.7	Scatter plot of body weight and wing length	27
4.1	PCR gel picture for <i>cytb</i> gene amplification	44
4.2	Mismatch distribution of the mitochondrial <i>cytb</i> haplotypes of <i>A. fuciphagus</i> .	44
4.3	Median Joining Network constructed based on the haplotypes obtained for <i>cyt b</i> gene locus of <i>A. fuciphagus</i>	45
4.4	Phylogenetic trees using Neighbour Joining method for the MtDNA <i>cytb</i> locus of 28 <i>A. fuciphagus</i> using Tamura – Nei 3 parameter method	46
4.5	Neighbour Joining phylogenetic trees based on the haplotypes of MtDNA <i>cytb</i> locus in <i>A. fuciphagus</i>	47
4.6	PCR gel picture for ND2 gene amplification	52
4.7	Mismatch distribution of the mitochondrial <i>ND2</i> haplotypes of <i>A. fuciphagus</i>	52
4.8	Median Joining Network constructed based on the haplotypes obtained for <i>ND2</i> gene locus of <i>A. fuciphagus</i>	53
4.9	Neighbour Joining, phylogenetic tree for the MtDNA <i>ND2</i> locus of 28 <i>A. fuciphagus</i> using Tamura – Nei 3 parameter method	54
4.10	Neighbour Joining, Phylogenetic trees based on the MtDNA <i>ND2</i> gene locus of 8 haplotypes used in this study and <i>A. fuciphagus</i> retrieved from the GenBank	55
4.11	PCR gel picture for <i>ND5</i> gene amplification	57
4.12	Mismatch distribution of the mitochondrial <i>ND5</i> haplotypes of <i>A. fuciphagus</i>	57
4.13	Neighbour Joining phylogenetic trees, for the MtDNA <i>ND5</i> locus of 28 <i>A. fuciphagus</i> using Kimura 2 parameter model	60
4.14	Neighbour Joining phylogenetic trees based on the MtDNA <i>ND5</i> gene locus of 5 haplotypes used in this study and other sequence retrieved from GenBank	61
4.15	Median Joining Network constructed based on the haplotypes obtained for <i>ND5</i> gene locus of <i>A. fuciphagus</i>	62
4.16	PCR gel picture for <i>ND3</i> gene amplification	63
4.17	PCR gel picture for <i>16SrRNA</i> gene amplification	64

4.18	Mismatch distribution for the mitochondrial <i>16S rRNA</i> haplotypes of <i>A. fuciphagus</i>	65
4.19	Median Joining Network constructed for the haplotypes obtained from <i>16S rRNA</i> gene locus of <i>A. fuciphagus</i>	68
4.20	Neighbour Joining phylogenetic trees for the mtDNA <i>16SrRNA</i> locus of 28 <i>A. fuciphagus</i> using Kimura 2 parameter model	69
4.21	Neighbour Joining phylogenetic trees for the mtDNA <i>16S rRNA</i> gene locus of six haplotypes used in this study with other species retrieved from the GenBank	70
4.22	PCR gel picture for <i>12SrRNA</i> gene amplification.	72
4.23	Mismatch distribution for the mitochondrial <i>12S rRNA</i> haplotypes of <i>A. fuciphagus</i>	72
4.24	Median Joining Network constructed for the haplotypes obtained from <i>12S rRNA</i> gene locus of <i>A. fuciphagus</i>	75
4.25	Neighbour Joining phylogenetic trees for the mtDNA <i>12srRNA</i> locus of 28 <i>A. fuciphagus</i> using Kimura 2 parameter model	76
4.26	Neighbour Joining phylogenetic trees for the mtDNA <i>12SrRNA</i> gene locus of five haplotypes and <i>A. fuciphagus</i> retrieved from GenBank	77
4.27	PCR gel picture for <i>COI</i> gene amplification	79
4.28	Mismatch distribution of the mitochondrial <i>COI</i> haplotypes of <i>A. fuciphagus</i>	79
4.29	Neighbour Joining phylogenetic trees for the mtDNA <i>COI</i> locus of 28 <i>A. fuciphagus</i> using Kimura 2 parameter model	82
4.30	Neighbour Joining tree for the mtDNA <i>COI</i> gene locus of four haplotypes and sequences retrieved from GenBank	83
4.31	Median Joining Network constructed for the haplotypes obtained from <i>COI</i> gene locus of <i>A. fuciphagus</i>	84
4.32	Dendogram of the six populations based on the seven mtDNA loci generated using neighbour joining method assessed at 1000 simulations	88

LIST OF ABBREVIATIONS

10X	ten times
1X	one time
A	Adenosine
BL	beak length
BT	beak thickness
Bp	base pair
C	cytosine
COI	cytochrome c oxidase 1
<i>Cytb</i>	cytochrome b
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EBN	edible bird nest
G	guanosine
GM	Gua Madai
Kb	kilobase
KG	Kota Tinggi
KT	Kuala Terengganu
LL	leg length
M	molar
MgCl ₂	magnesium chloride
mM	milimolar
<i>ND2</i>	NADH dehydrogenase subunit-2
<i>ND3</i>	NADH dehydrogenase subunit-3
<i>ND5</i>	NADH dehydrogenase subunit-5

PCR	polymerase chain reaction
PT	Pitas
RNA	ribonucleic acid
rpm	revolution per minute
SP	Sungkai
T	Thymine
TAE	tris-acetic-EDTA
TM	Tanah Merah
TL	tail length
UV	ultraviolet
W	Watt
WL	wing length
<i>16S rRNA</i>	16s ribosomal RNA
<i>12S rRNA</i>	12s ribosomal RNA

CHAPTER 1

INTRODUCTION

There are over 24 echolocating species of swiftlets from the genus *Aerodramus* but only a few produce edible nests of good qualities around the world (Marcone, 2005). The species of swiftlet known to be the highest producers of bird nest traded in the world are the white nest swiftlet (*Aerodramus fuciphagus*) and the Black nest swiftlet (*Aerodramus maximus*). Both species are found in large populations in the East Borneo and Peninsular Malaysia.

Aerodramus fuciphagus are small sized insectivorous swifts that inhabit limestone caves of many parts of South East Asia (Chantler *et al.*, 1999; Chantler & Driessens, 2000; Marcone, 2005) and are first class flyers flying at high velocity (Videler *et al.*, 2004). They possess the ability to echolocate, a characteristic shared only by Neotropical oilbird (Steatornithidae) (Suthers & Hector, 1985; Price *et al.*, 2004). In Malaysia they are known as “Burung Walit”. These birds are unique in that they produce nests that are deemed edible and are of commercial importance. This edible nest swiftlet (EBN) builds nests wholly of the mucilaginous secretion from the pair of sublingual salivary glands, intricately constructed without feather, moss or twigs (Marshall & Folley, 2009).

EBNs are highly sought after due to their nutritional, aphrodisiac and medicinal properties (Goh *et al.*, 2001; Sankaran, 2001; Lim, 2002; Guo *et al.*, 2006; Norhayati *et al.*, 2010). Recently, local EBN industries have ventured into providing artificial housing for these swiftlets and producing house EBNs because of the commercial value of the nests. This has increased the number of birds, but may in the long term have an adverse effect on the overall population of swiftlets (Chantler & Driessens, 2000; Sankaran, 2001; Marcone, 2005; Aowphol *et al.*, 2008;). The EBN swiftlet is a unique species and in the attempts to increase their numbers, due consideration has to be given to maintaining the genetic stability of the populations. Due to its ever increasing demand, the survival of the EBN swiftlets lies in a pragmatic approach to the exploitation of its nest (Sankaran, 2001). As such, this highly placed species needs correct management with genetic conservation as one of the priorities for enhancement of the population and the EBN industry.

Population genetic studies of swiftlet are important in both the natural and man-made colonies for continual survival of the species. Genetic diversity data is needed to monitor the genetic variability of species as loss of diversity is a treat to the survival of a population (Frankham, 2003). The advances in molecular techniques and genetic analysis enable utilisation of molecular markers to access pools of genetic variability (Avise, 1994).

Limited works have been done on the EBN swiftlets in Malaysia to date, and consequently, limited literature is available. In addition, few publications are available on the genetics and morphometry makeup of the natural EBN swiftlet populations. There is also no adequate knowledge about the delineation among swiftlet species in Malaysia. These make it difficult to evaluate genetic variation and the effects of the growing industry on the local swiftlet populations. Since the qualities of the EBNs determine the price and the demand, there is also a demand for a traceability system. DNA barcode is ideal for this purpose; if established it may be used to identify origin of a genetic material.

1.1 Objectives

This study was aimed to assess mtDNA genes and elucidate the genetic variability of *A. fuciphagus* in Malaysia.

The specific objectives of this study were:

1. to assess the morphometric variations of the EBN swiftlet populations in Perak, Terengganu and Kelantan.
2. to evaluate the genetic variation among EBN swiftlets from different locations in Malaysia based on seven mtDNA loci.
3. to identify DNA barcodes for EBN swiftlet to facilitate traceability by location.

REFERENCES

- Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71(1), 5-16.
- Aliabadian, M., Kaboli, M., Prodon, R., Nijman, V., & Vences, M. (2007). Phylogeny of Palearctic wheatears (genus *Oenanthe*) Congruence between morphometric and molecular data. *Molecular Phylogenetics and Evolution*, 42(3), 665-675.
- Anne, C. (2006). Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. *Genetica*, 127 (13), 101-120.
- Aowphol, A., Voris, H. K., Feldheim, K. A., Harnyuttanakorn, P., & Thirakhupt, K. (2008). Genetic Homogeneity Among Colonies of the White-Nest Swiftlet (*Aerodramus fuciphagus*) in Thailand. *Zoological Science*, 25(4), 372-380.
- AOU, (1998). *The AOU Check-list of North American Birds, 7th edition*. The American Ornithologists' Union, Washington, D.C
- Avise, J. C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.
- Avise, J. C. (2000). *Phylogeography: the History and Formation of Species*. Harvard University Press.
- Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Saunders, N. C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, 18, 489-522.
- Avise, J. C., & Walker, D. (1999). Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. *Proceedings of the National Academy of Sciences*, 96(3), 992-995.
- Avise J. C., & Zink, R.M (1988). Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boated-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. *Auk* 105: 516-528.
- Bagherian, A., & Rahmani, H. (2007). Morphological differentiation between two populations of the Shemaya, *Chalcalburnus chalcoides*: a geometrical morphometric approach. *Zoology in the Middle East*, 40(1), 53-62.
- Bagherian, A., & Rahmani, H. (2009). Morphological discrimination between two populations of shemaya, *Chalcalburnus chalcoides* (Actinopterygii,

Cyprinidae), using a truss network. *Animal Biodiversity and Conservation*, 32(1), 1-8.

- Baldassarre, D. T., Thomassen, H. A., Karubian, J., & Webster, M. S. (2013). The role of ecological variation in driving divergence of sexual and non-sexual traits in the red-backed fairy-wren (*Malurus melanocephalus*). *BMC Evolutionary Biology*, 13(1), 75.
- Baker, K. Identification Guide to European Non-Passerines: BTO Guide 24, 1993. *British Trust for Ornithology, Thetford, UK*.
- Balitzki-Korte, B., Anslinger, K., Bartsch, C., & Rolf, B. (2005). Species identification by means of pyrosequencing the mitochondrial 12S rRNA gene. *International Journal of Legal Medicine*, 119(5), 291-294.
- Banks, R. C., Cicero, C., Dunn, J. L., Kratter, A. W., Ouellet, H., Rasmussen, P. C., & Stotz, D. F. (2000). Forty-second supplement to the American Ornithologists' Union check-list of North American birds. *The Auk*, 117, 847-858.
- Banks, R. C., Cicero, C., Dunn, J. L., Kratter, A. W., Rasmussen, P. C., Remsen Jr, J. V., & Stotz, D. F. (2002). Forty-second supplement to the Ornithologists' Union checklist of North American birds. *The Auk*, 119(3), 897-906.
- Banks, R. C., Cicero, C., Dunn, J. L., Kratter, A. W., Rasmussen, P. C., Remsen Jr, J. V., & Stotz, D. F. (2002). Fortythird supplement to the Ornithologists' Union checklist of North American birds. *The Auk*, 119(3), 897-906.
- Banks, R. C., Cicero, C., Dunn, J. L., Kratter, A. W., Rasmussen, P. C., Remsen Jr, J. V., & Stotz, D. F. (2003). Fortyfourth supplement to the Ornithologists' Union checklist of North American birds. *The Auk*, 120(3), 923-931.
- Bensch, S., & Hasselquist, D. (1999). Phylogeographic population structure of great reed warblers: an analysis of mtDNA control region sequences. *Biological Journal of the Linnean Society*, 66(2), 171-185.
- Brooke, R. (1970). Taxonomic and evolutionary notes on the subfamilies, tribes, genera and subgenera of the swifts (Aves: Apodidae). *Durban Mus. Novit*, 9(2), 13-24.
- Brooke, R. K. (1972). Generic limits in old world Apodidae and Hirundinidae. *Bulletin of the British Ornithologists' Club*, 92, 53-57.
- Bookstein, E. L. (1991). *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, London.
- Buerkle, C. A. (1999). The historical pattern of gene flow among migratory and non-migratory populations of prairie warblers Aves: Parulinae. *Evolution*, 53, 1915-1924.

- Bull, L. S., Haywood, J., & Pledger, S. (2004). Components of phenotypic variation in the morphometrics of shearwater (*Puffinus*) species. *Ibis*, *146*(1), 38-45.
- Burg, T. M., & Croxall, J. P. (2001). Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology*, *10*(11), 2647-2660.
- Burns, K. J., & Racicot, R. A. (2009). Molecular phylogenetics of a clade of lowland tanagers: implications for avian participation in the Great American Interchange. *The Auk*, *126*(3), 635-648.
- Camfield, A. (2004). Apodidae. *Animal Diversity Web (online)*. Accessed February 13, 2014 at <http://animaldiversity.ummz.umich.edu/accounts/Apodidae/>
- Campbell, A. K. (2003). Save those molecules! Molecular biodiversity and life. *Journal of Applied Ecology*, *40*(2), 193-203.
- Cavalcanti, M. J., Monteiro, L. R., & Lopes, P. R. (1999). Landmark-based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). *Zoological Studies Taipei*, *38*(3), 287-294.
- Cespedes, A., Garcia, T., Carrera, E., Gonzalez, I., Sanz, B., Hernaz, P. E., & Martin, R. (1998). Identification of flatfish species using polymerase chain reaction (PCR) amplification and restriction analysis of the cytochrome b gene. *Journal of Food Science*, *63*(2), 206-209.
- Chaney Jr, R. C., Blemings, K. P., Bonner, J., & Klandorf, H. (2003). Pentosidine as a measure of chronological age in wild birds. *The Auk*, *120*(2), 394-399.
- Chantler, P. (1995). *Swifts. A Guide to the Swifts and Treeswifts of the World*: New York: Pica Press.
- Chantler, P., & Driessens, G. (2000). *Swifts: A Guide to the Swifts and Treeswifts of the World*. A&C Black.
- Chantler, P., Wells, D., & Schuchmann, K. (1999). Family Apodidae (Swifts). *Handbook of the Birds of the World*, *5*, 388-457.
- Chesser, R. T. (1999). Molecular systematics of the rhinocryptid genus *Pteroptochos*. *Condor*, *101*, 439-446.
- Chikhi, L., Goossens, B., Treanor, A., & Bruford, M. (2004). Population genetic structure of and inbreeding in an insular cattle breed, the Jersey, and its implications for genetic resource management. *Heredity*, *92*(5), 396-401.
- Clare, E. L., Lim, B. K., Engstrom, M. D., Eger, J. L., & Hebert, P. D. (2007). DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes*, *7*(2), 184-190.

- Clements R, Sodhi NS, Schilthuizen M, Ng PK (2006) Limestone katsts of Southeast Asia: imperiled arks of biodiversity. *Bio- Science* 56: 733–742
- Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist*, 661-696.
- Cranbrook, E. O., Lim, G. W., Koon, L. C., & Rahman, M. A. (2013). The species of white-nest swiftlets (Apodidae, Collocaliini) of Malaysia and the origins of house-farm birds: morphometric and genetic evidence. *Forktail*, (29), 107-119.
- Crandall, K. A., Bininda-Emonds, O. R., Mace, G. M., & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, 15(7), 290-295.
- Davis, L. A., Roalson, E. H., Cornell, K. L., Mcclanahan, K. D., & Webster, M. S. (2006). Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. *Molecular Ecology*, 15(8), 2141-2152.
- Degli Esposti, M., Ghelli, A., Ratta, M., Cortes, D., & Estornell, E. (1994). Natural substances (acetogenins) from the family Annonaceae are powerful inhibitors of mitochondrial NADH dehydrogenase (Complex I). *Biochemical Journal*, 301, 161-167.
- Desjardins, P., & Morais, R. (1990). Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. *Journal of Molecular Biology*, 212(4), 599-634.
- Di Finizio, A., Guerriero, G., Russo, G. L., & Ciarcia, G. (2007). Identification of gadoid species (Pisces, Gadidae) by sequencing and PCR–RFLP analysis of mitochondrial 12S and 16S rRNA gene fragments. *European Food Research and Technology*, 225(3-4), 337-344.
- Dmitrenok, M., Puglisi, L., Demongin, L., Gilbert, G., Polak, M., & Bretagnolle, V. (2007). Geographical variation, sex and age in Great Bittern *Botaurus stellaris* using coloration and morphometrics. *Ibis*, 149(1), 37-44.
- Dove, C. J. (2000). A descriptive and phylogenetic analysis of plumulaceous feather characters in Charadriiformes. *Ornithological Monographs*, 51, 1-163.
- Dujardin, J.-P., & GEMI, I. (2011). Modern morphometrics of medically important insects. *Genetics and Evolution of Infectious Diseases*, 473-501.
- Fenton, M. B. (1975). Observations on the biology of some Rhodesian bats, including a key to the Chiroptera of Rhodesia. *Life Sciences Contributions, Royal Ontario Museum*. 104, 1-27.

- Féral, J.-P. (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity? *Journal of Experimental Marine Biology and Ecology*, 268(2), 121-145.
- Fitpatrick S., (1997). Patterns of morphometric variation in birds' tails: length, shape and variability. *Biological Journal of the Linnean Society*, 62(1), 145- 162.
- Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biologies*, 326, 22-29.
- Frazer, K.A., Elnitski, L., Deanna, M. C., Dubchak, I. and Hardison R.C. (2003). Cross-species sequence comparisons: A review of methods and available resources. *Genome Research* 13:1–12.
- Freeman, S., & Jackson, W. M. (1990). Univariate metrics are not adequate to measure avian body size. *The Auk*, 107, 69-74.
- Frézal, L., & Leblois, R. (2008). Four years of DNA barcoding: current advances and prospects. *Infection, Genetics and Evolution*, 8(5), 727-736.
- Fullard, J. H., Barclay, R. M., & Thomas, D. W. (1993). Echolocation in free-flying Atiu swiftlets (*Aerodramus sawtelli*). *Biotropica*, 25, 334-339.
- Galtier, N., Nabholz, B., Glémin, S., & Hurst, G. (2009). Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, 18(22), 4541-4550.
- Garcia-Moreno, J., Arctander, P., & Fjeldså, J. (1999). Strong diversification at the treeline among *Metallura* hummingbirds. *The Auk*, 116, 702-711.
- Gausset, Q. (2004). Chronicle of a foreseeable tragedy: birds' nests management in the niah caves (Sarawak). *Human Ecology*, 32(4), 487-507.
- Gill, F. B., & Slikas, B. (1992). Patterns of mitochondrial DNA divergence in North American crested titmice. *Condor*, 94, 20-28.
- Girish, P., Anjaneyulu, A., Viswas, K., Anand, M., Rajkumar, N., Shivakumar, B., & Bhaskar, S. (2004). Sequence analysis of mitochondrial 12S rRNA gene can identify meat species. *Meat Science*, 66(3), 551-556.
- Gissi, C., Iannelli, F., & Pesole, G. (2008). Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. *Heredity*, 101(4), 301-320.
- Goh, D. L. M., Chua, K. Y., Chew, F. T., Liang, R. C. M. Y., Seow, T. K., Ou, K. L., & Lee, B. W. (2001). Immunochemical characterization of edible bird's nest allergens. *Journal of Allergy and Clinical Immunology*, 107(6), 1082-1088.

- Granadeiro, J. P. (1991 b). On a Cory's Shearwater ringed at Selvagem Grande, Madeira (30°09'N, 15°52'W) and recovered on Berlenga island, Portugal (39°24'N, 93°0'W). *Bocagiana*, 145, 4.
- Grant, V., & Grant, K. A. (1979). The pollination spectrum in the southwestern American cactus flora. *Plant Systematics and Evolution*, 133(1-2), 29-37.
- Groeneveld Le, F., Lenstra J.A, Eding H, Toro M.A, Scherf B, Pilling D, Negrini R, Finlay E.K, Jianlin H, Groeneveld E, Weigeng, and the GLOBALDIV Consortium. (2010). Genetic diversity in farm animals - a review. *International Society for Animal Genetics*, 41(1), 6 - 31.
- Guo, C.-T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., Suzuki, Y. (2006). Edible bird's nest extract inhibits influenza virus infection. *Antiviral Research*, 70(3), 140-146.
- Guglich, E. A., Wilson, P. J., & White, B. N. (1994). Forensic application of repetitive DNA markers to the species identification of animal tissues. *Journal of Forensic Sciences*, 39, 353-353.
- Haig, S., Gratto-Trevor, C., Mullins, T., & Colwell, M. (1997). Population identification of western hemisphere shorebirds throughout the annual cycle. *Molecular Ecology*, 6(5), 413-427.
- Harper, J. L., Lovell, P. H., & Moore, K. G. (1970). The shapes and sizes of seeds. *Annual Review of Ecology and Systematics*, 1: 327-356.
- Hebert, P. D., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270 (Suppl 1), S96-S99.
- Hebert, P. D., Stoeckle, M. Y., Zemplak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS Biology*, 2(10), e312.
- Helbig, A., Salomon, M., Bensch, S., & Seibold, I. (2001). Male-biased gene flow across an avian hybrid zone: evidence from mitochondrial and microsatellite DNA. *Journal of Evolutionary Biology*, 14(2), 277-287.
- Hillel, J., Granevitze, Z., Twito, T., Ben-Avraham, D., Blum, S., Lavi, U., & Weigend, S. (2007). Molecular markers for the assessment of chicken biodiversity. *World's Poultry Science Journal*, 63(01), 33-45.
- Hudson, R. R., & Turelli, M. (2003). Stochasticity overrules the “three-times rule”: genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. *Evolution*, 57(1), 182-190.

- Hurst, G. D., & Jiggins, F. M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society B: Biological Sciences*, 272(1572), 1525-1534.
- Irwin, D. M., Kocher, T. D., & Wilson, A. C. (1991). Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution*, 32(2), 128-144.
- Iwaniuk, A. N., Clayton, D. H., & Wylie, D. R. (2006). Echolocation, vocal learning, auditory localization and the relative size of the avian auditory midbrain nucleus (MLd). *Behavioural Brain Research*, 167(2), 305-317.
- Johnsen, A., Rindal, E., Ericson, P. G., Zuccon, D., Kerr, K. C., Stoeckle, M. Y., & Lifjeld, J. T. (2010). DNA barcoding of Scandinavian birds reveals divergent lineages in trans-Atlantic species. *Journal of Ornithology*, 151(3), 565-578.
- Johnson, C. G. (1969). Migration and dispersal of insects by flight. *Migration and dispersal of insects by flight*. Methuen, London.
- Kerr, K. C., Stoeckle, M. Y., Dove, C. J., Weigt, L. A., Francis, C. M., & Hebert, P. D. (2007). Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes*, 7(4), 535-543.
- Koch, H. (2010). Combining morphology and DNA barcoding resolves the taxonomy of western Malagasy *Liotrigona* Moure, 1961 (Hymenoptera: Apidae: Meliponini). *African Invertebrates*, 51(2), 413-421.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, 86(16), 6196-6200.
- Koon, L. C. Cranbrook, Earl of (2002). *Swiftlets of Borneo. Builders of Edible Nests*. Natural History Publication, Kota Kinabalu, Borneo.
- Koon, L. C., & Cranbrook Earl of, 2002. *Swiftlets of Borneo: Builders of Edible Nests*. Kota Kinabalu: Natural History Publication (Borneo).
- Laiolo, P., & Rolando, A. (2001). Ecogeographic correlates of morphometric variation in the Red-billed Chough *Pyrrhocorax pyrrhocorax* and the Alpine Chough *Pyrrhocorax graculus*. *Ibis*, 143(3), 602-616.
- Langham, N. (1980). Breeding Biology of the Edible Bird Nest Swiftlet *Aerodramus fuciphagus*. *Ibis*, 122(4), 447-461.
- Langridge, P., & Chalmers, K. (2005). The principle: identification and application of molecular. *Springer*, 55, 3-22.
- Lau, A.S.M., Melville, S., 1994. International Trade in Swiftlet Nests with Special Reference to Hong Kong. TRAFFIC International, Cambridge.

- Lee, J. H., Hassan, H., Hill, G., Cupp, E. W., Higazi, T. B., Mitchell, C. J., ... & Unnasch, T. R. (2002). Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. *The American Journal of Tropical Medicine and Hygiene*, 66(5), 599.
- Lee, P., Clayton, D. H., Griffiths, R., & Page, R. (1996). Does behavior reflect phylogeny in swiftlets (Aves: Apodidae)? A test using cytochrome b mitochondrial DNA sequences. *Proceedings of the National Academy of Sciences*, 93(14), 7091-7096.
- Lim, C. K., Cranbrook, G. G. H., Cranbrook, G. G. H., Zoologiste, G. B., Cranbrook, G. G. H., & Zoologist, G. B. (2002). *Swiftlets of Borneo: Builders of Edible Nests*. Natural History Publications (Borneo).
- Lindman, H. R. (1992). *Analysis of Variance in Experimental Design*. Springer-Verlag Publishing.
- Lockley, A., & Bardsley, R. (2000). DNA-based methods for food authentication. *Trends in Food Science and Technology*, 11(2), 67-77.
- Lourie, S.A., & Tompkins, D. M. (2000). The diets of Malaysian swiftlets. *Ibis*, 142(4), 596-602.
- Mammadov, J., Aggarwal, R., Buyyarapu, R. and Kumpatla, S. (2012). SNP markers and their impact on plant breeding. *International Journal of Plant Genomics*,
- Manchi, S. S., & Sankaran, R. (2010). Foraging Habits and Habitat Use by Edible-nest and Glossy Swiftlets in the Andaman Islands, India. *The Wilson Journal of Ornithology*, 122(2), 259-272.
- Marcus, L. F. (1990). Traditional morphometrics. *Proceedings of the Michigan Morphometrics Workshop*. University of Michigan Museum of Zoology, Ann Arbor. 2, 77 – 122.
- Mardiastuti, A., & Mranata, B. (1996). *Biology and distribution of Indonesian swiftlets with a special reference to Collocalia fuciphaga and Collocalia maxima*. Paper presented at the The Technical Workshop on Conservation Priorities and Actions for the Sustainability of Harvesting and Trade Nest of Swiftlets of the Genus Collocalia that Feature Prominently in The Bird Nest Trade.
- Mardiastuti, A., Mulyani, Y. A., & Gultom, T. A. (1997). Breeding success of Edible-nest Swiftlets in a man-made nesting habitat. *Media Konservasi*, 5(2)
- Marshall, A., & Folley, S. (2009). The Origin of Nest-Cement in Edible Bird Nest Swiftlets (*Collocalia* spp). *Proceedings of the Zoological Society of London*. 126(3), 383-390.

- Mather, D. D., & Esler, D. (1999). Evaluation of Bursal Depth as an Indicator of Age Class of Harlequin Ducks. *Journal of Field Ornithology*, 70, 200-205.
- Matthee, C. A., & Flemming, A. F. (2002). Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. *Molecular Ecology*, 11(3), 465-471.
- Mayr, E. (1937). Notes on the Birds 'of Northern Melanesia. 3 'Passeres. *Journal of Washington Academic Science*, 37, 95-98.
- Mayr, E., & Rand, A. L. (1937). The birds of the 1933-1934 Papuan Expedition. Bulletin of the AMNH; v. 73, article 1.
- Medway, L. (1962). The swiftlets (Collocalia) of Niah Cave, Sarawak. *Ibis*, 104(1), 45-66.
- Medway, L. (1967). The function of echolocation among swiftlets. *Animal Behaviour*, 15(4), 416-420.
- Melton, T., & Holland, C. (2007). Routine forensic use of the mitochondrial 12S ribosomal RNA gene for species identification. *Journal of Forensic Sciences*, 52(6), 1305-1307.
- Meyer, A. (1994). Shortcomings of the cytochrome gene as a molecular marker. *Trends in Ecology and Evolution*, 9(8), 278-280.
- Michael, E., Ramaiah, K. D., Hoti, S. L., Barker, G., Paul, M. R., Yuvaraj, J., & Bundy, D. A. P. (2001). Quantifying mosquito biting patterns on humans by DNA fingerprinting of bloodmeals. *American Journal of Tropical Medicine and Hygiene*, 65(6), 722-728.
- Miller, S. L., Gregg, M. A., Kuritsubo, A. R., Combs, S. M., Murdock, M. K., Nilsson, J. A., Botzler, R. G. (1988). Morphometric variation in Tundra Swans: relationships among sex and age classes. *Condor*, 90, 802-815.
- Mindell, D. P., & Honeycutt, R. L. (1990). Ribosomal RNA in vertebrates: evolution and phylogenetic applications. *Annual Review of Ecology and Systematics*, 21, 541-566.
- Mindell, D. P., Sorenson, M. D., Huddleston, C. J., Miranda Jr, H. C., Knight, A., Sawchuk, S. J., & Yuri, T. (1997). Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. *Avian Molecular Evolution and Systematics*, 213-247.
- Mitani, T., Akane, A., Tokiyasu, T., Yoshimura, S., Okii, Y., & Yoshida, M. (2009). Identification of animal species using the partial sequences in the mitochondrial 16S rRNA gene. *Legal Medicine*, 11, S449-S450.
- Mohaddasi, M., Shabanipour, N., & Abdolmaleki, S. (2013). Morphometric variation among four populations of Shemaya (*Alburnus chalcoides*) in the south of

- Caspian Sea using truss network. *The Journal of Basic and Applied Zoology*, 66(2), 87-92.
- Morgan, J.H. (2004). Remarks on the taking and recording of biometric measurements in birds ringing. *Ring*, 26, 71-78, ISSN 0035-5429
- Morin, P. A., Luikart, G., & Wayne, R. K. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology and Evolution*, 19(4), 208-216.
- Moritz, C., & Cicero, C. (2004). DNA barcoding: promise and pitfalls. *PLoS Biology*, 2(10), e354.
- Moore, W. S. (1995). Inferring phylogenies from mtDNA variation: Mitochondrial gene trees versus nuclear-gene trees. *Evolution*, 49, 718–726.
- Mueller, H. C. (1990). Can Saw-Whet Owls be Sexed by External Measurements. *Journal of Field Ornithology*, 339-346.
- Murray, B.W., McGillivray, W.B., Barlow, J.C., Beech, R.N, & Strobeck, C. (1994) The use of cytochrome B sequence variation in estimation of phylogeny in the Vireonidae. *Condor* 96: 1037–1054.
- Nakamura, H. (2009). Forensic species identification based on size variation of mitochondrial DNA hypervariable regions. *International Journal of Legal Medicine*, 123(2), 177-184.
- Nakamura, M., Ishibashi, Y., & Syuiti, A. (2002). Novel primer sets for species-specific amplification of the mitochondrial 12S rRNA genes in four Japanese woodpeckers (Picidae, Piciformes). *Molecular Ecology Notes*, 2(4), 419-421.
- Nesje, M., Røed, K., Bell, D., Lindberg, P., & Lifjeld, J. (2000). Microsatellite analysis of population structure and genetic variability in peregrine falcons (*Falco peregrinus*). *Animal Conservation*, 3(3), 267-275.
- Nevo, E. (2001). Evolution of genome–phenome diversity under environmental stress. *Proceedings of the National Academy of Sciences*, 98(11), 6233-6240.
- Norberg, U. M. (1986). Evolutionary convergence in foraging niche and flight morphology in insectivorous aerial-hawking birds and bats. *Ornis Scandinavica*, 253-260.
- Norhayati, M., Azman, O., & Wan Nazaimoon, W. (2010). Preliminary study of the nutritional content of Malaysian edible bird's nest. *Malaysian Journal of Nutrition*, 16(3), 389-396.
- Nugroho, E., & Whendrato, I. (1996). *The farming of edible-nest swiftlets in Indonesia*. Paper presented at the Technical Workshop on Conservation Priorities and Actions for Edible-nest Swiftlets, Surabaya, Indonesia.

- Olsson, U., Alström, P., & Sundberg, P. (2004). Non-monophyly of the avian genus *Seicercus* (Aves: Sylviidae) revealed by mitochondrial DNA. *Zoologica scripta*, 33(6), 501-510.
- Ouborg, N., Piquot, Y., & Van Groenendael, J. (1999). Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology*, 87(4), 551-568.
- Parson, W., Pegoraro, K., Niederstätter, H., Föger, M., & Steinlechner, M. (2000). Species identification by means of the cytochrome b gene. *International Journal of Legal Medicine*, 114(1-2), 23-28.
- Paton, T. A., & Baker, A. J. (2006). Sequences from 14 mitochondrial genes provide a well-supported phylogeny of the charadriiform birds congruent with the nuclear RAG-1 tree. *Molecular Phylogenetics and Evolution*, 39(3), 657-667.
- Paulo, O. S., Pinto I., Bruford, M. W., Jordan, W. C & Nichols, R. A. (2002). The double origin of Iberian peninsular chameleons. *Biological Journal of the Linnean Society* 75, 1-7.
- Pichorim, M (2010). Influence of age and season on morphometric measurements of the Biscutate Swift (*Streptoprocne biscutata*).. (n.d.) >*The Free Library*. (2014). Retrieved Aug 13 2014 from <http://www.thefreelibrary.com/Influence+of+age+and+season+on+morphometric+measurements+of+the...-a0221917693>
- Pichorim, M., & Monteiro-Filho, E. (2008). Brood size and its importance for nestling growth in the Biscutate Swift (*Streptoprocne biscutata*, Aves: Apodidae). *Brazilian Journal of Biology*, 68(4), 851-857.
- Pothieng D (2005) Ecology and distribution of white-nest swiftlet (*Collocalia fuciphagus*). *Compilation of 2004 Research, Progressive Reports and Essays of Wildlife Ecology, Wildlife Research Division, Natural Park, Wildlife and Plans Conservation Department, Bangkok*, 89–113
- Poulet, N., Berrebi, P., Crivelli, A. J., Lek, S., & Argillier, C. (2004). Genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented delta. *Archiv für Hydrobiologie*, 159(4), 531-554.
- Prakash, S., Patole, M., Ghumatkar, S., Nandode, S., Shinde, B., & Shouche, Y. (2000). Mitochondrial 12S rRNA sequence analysis in wildlife forensics. *Current Science*, 78(10), 1239-1241.
- Price, J.J., Johnson, K.P., & Clayton, D.H. (2004). The evolution of echolocation in swiftlets. *Journal of Avian Biology*, 35(2), 135-143.
- Primmer, C. R., Raudsepp, T., Chowdhary, B. P., Møller, A. P., & Ellegren, H. (1997). Low frequency of microsatellites in the avian genome. *Genome Research*, 7(5), 471-482.

- Prince, P. A., & Rodwell, S. P. (1994). Aging immature black-browed and grey-headed albatrosses using moult, bill and plumage characteristics. *Emu*, 94(4), 246-254.
- Pyle, P. (1997). Identification guide to North American birds. Part I. Columbidae to Ploceidae. Slate Creek Press, Bolinas, California
- Quang, P. N., Quang, Y. V., Voisin, J.-F., Suiro, P. L., & Candiard, J. (2002). *The White-nest Swiftlet and the Black-nest Swiftlet: A monograph*: Société nouvelle des éditions Boubée.
- Rastogi, G., Dharne, M., Bharde, A., Pandav, V., Ghumatkar, S., Krishnamurthy, R., Shouche, Y. S. (2004). Species determination and authentication of meat samples by mitochondrial 12S rRNA gene sequence analysis and conformation-sensitive gel electrophoresis. *Current Science Bangalore* , 87, 1278-1281.
- Rieseberg, L. H., & Burke, J. M. (2001). The biological reality of species: gene flow, selection, and collective evolution. *Taxon* 50, 47-67.
- Roeder, A. D., Marshall, R. K., Mitchelson, A. J., Visagathilagar, T., Ritchie, P. A., Love, D. R., & Lambert, D. M. (2001). Gene flow on the ice: genetic differentiation among Adelie penguin colonies around Antarctica. *Molecular Ecology*, 10(7), 1645-1656.
- Rohlf, F. J. (1990). Morphometrics. *Annual Review of Ecology and Systematics*, 21, 299-316.
- Rohlf, F. J., & Marcus, L. F. (1993). A revolution morphometrics. *Trends in Ecology and Evolution*, 8(4), 129-132.
- Salomonsen, F. (1983). Revision of the Melanesian Swiftlets (Apodes, Aves) and their conspecific forms in the Indo-Australian and Polynesian region. *Biologiske Skrifter Kongelige Danske Videnskabernis Selskab*, 23(5), 1-112.
- Sankaran, R. (2001). The status and conservation of the Edible-nest Swiftlet (*Collocalia fuciphaga*) in the Andaman and Nicobar Islands. *Biological Conservation*, 97(3), 283-294.
- Sankaran R. Manchi S. (2008) Conservation of the Edible-nest Swiftlets in the Andaman and Nicobar Islands. Sálím Ali Centre for Ornithology and Natural History, Report to the Department of Environment and Forest, Andaman and Nicobar Islands.
- Shyamal, L. (2007). Morphometric measurements of birds are important in systematics. The wikimedia common. (2014). Retrieved Sept 24 2014 from <http://en.wikipedia.org/wiki/Ornithology#mediaviewer/File:BirdMorpho.svg>.
- Sibley, C. G., & Monroe, B. L. (1990). *Distribution and Taxonomy of Birds of the World*: Yale University Press.

- Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America*, 103(10), 3657-3662.
- Smythies, B. E. 1981. *The birds of Borneo. Third Edition*. Sabah Society and Malayan Nature Society. Kuala Lumpur, Malaysia.
- SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc
- Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, 15(5), 199-203.
- Suthers, R. A., & Hector, D. H. (1982). Mechanism for the production of echolocating clicks by the grey swiftlet, *Collocalia spodiopygia*. *Journal of Comparative Physiology*, 148(4), 457-470.
- Suthers, R. A., & Hector, D. H. (1985). The physiology of vocalization by the echolocating oilbird, *Steatornis caripensis*. *Journal of Comparative Physiology A*, 156(2), 243-266.
- Svensson, L. 1992. *Identification Guide to European Passerines*, 4th edn. Stockholm: L. Svensson.
- Swain, D. P., Riddell, B. E., & Murray, C. B. (1991). Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. *Canadian Journal of Fisheries and Aquatic Sciences*, 48(9), 1783-1791.
- Thomassen, H. A., Djasim, U. M., & Povel, G. D. E. (2004). Echoclick design in swiftlets: single as well as double clicks. *Ibis*, 146(1), 173-174.
- Thomassen, H. A., & Povel, G. D. E. (2006). Comparative and phylogenetic analysis of the echo clicks and social vocalizations of swiftlets (Aves: Apodidae). *Biological Journal of the Linnean Society*, 88(4), 631-643.
- Thomassen, H. A., Wiersema, A. T., de Bakker, M. A., de Knijff, P., Hetebrij, E., & Povel, G. D. E. (2003). A new phylogeny of swiftlets (Aves: Apodidae) based on cytochrome b DNA. *Molecular Phylogenetics and Evolution*, 29(1), 86-93.
- Tokarska, M., Marshall, T., Kowalczyk, R., Wójcik, J. M., Pertoldi, C., Kristensen, T. N., & Bendixen, C. (2009). Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: the case of European bison. *Heredity*, 103(4), 326-332.
- Vences, M., Thomas, M., Bonett, R. M., & Vieites, D. R. (2005). Deciphering amphibian diversity through DNA barcoding: chances and challenges.

Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462), 1859-1868.

- Videler, J., Stamhuis, E., & Povel, G. (2004). Leading-edge vortex lifts swifts. *Science*, 306(5703), 1960-1962.
- Vignal, A., Milan, D., Sancristobal, M. and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution*, 34, 275-305.
- Viruhpintu, S., Thirakhupt, K., Pradatsundarasar, A., & Poonswad, P. (2002). Nest-site Characteristics of the Edible-nest Swiftlet *Aerodramus fuciphagus* (Thunberg, 1812) at Si-Ha Islands, Phattalung Province, Thailand. *Natural History*, 2(2), 31-35.
- Weibel, A. C., & Moore, W. S. (2002). A Test of a Mitochondrial Gene-Based Phylogeny of Woodpeckers (Genus *Picoides*) Using an Independent Nuclear Gene, β -Fibrinogen Intron 7. *Molecular Phylogenetics and Evolution*, 22(2), 247-257.
- Welty, J. C., & Tolson, N. (1975). *The life of Birds*. Philadelphia: Saunders, 632.
- Wenink, P. W., A.J. Baker, H.-U. Rosner and M. G.J. Tilanus. (1996). Global mitochondrial DNA phylogeography of holarctic breeding Dunlins (*Calidris alpina*). *Evolution*(50), 318-330.
- Wennerberg, L. (2001). Breeding origin and migration pattern of dunlin (*Calidris alpina*) revealed by mitochondrial DNA analysis. *Molecular Ecology*, 10(5), 1111-1120.
- Willig, M. R., Owen, R. D., & Colbert, R. L. (1986). Assessment of morphometric variation in natural populations: the inadequacy of the univariate approach. *Systematic Biology*, 35(2), 195-203.
- Wimberger, P. H. (2008). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biological Journal of the Linnean Society*, 45(3), 197-218.
- Yang, S. J., Lei, F. M., Qu, Y. H., & Yin, Z. H. (2006). Intraspecific phylogeography of the white-rumped snowfinch (*Onychostruthus taczanowskii*) endemic to the Tibetan Plateau based on mtDNA sequences. *Journal of Zoology*, 268(2), 187-192.
- Yakubu, A., Kaankuka, F. G., & Ugbo, S. B. (2011). Morphometric traits of Muscovy ducks from two agro-ecological zones of Nigeria. *Tropicultura*, 29(2), 121-124.
- Yoo, H. S., Eah, J. Y., & Kim, J. S. 2006. DNA barcoding Korean birds. *Molecules and Cells*, 22(3), 323-327.

Zink, R. M., & Barrowclough, G. F. (2008). Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, 17(9), 2107-2121.

Zuki, A., Abdul Ghani, M., Khadim, K., Intan-Shameha, A., & Kamaruddin, M. (2012). Anatomical structures of the limb of white-nest swiftlet (*Aerodramus fuciphagus*) and white-headed munia (*Lonchura maja*). *Pertanika Journal of Tropical Agricultural Science*, 35(3), 613-622.



© COPYRIGHT UPM