



UNIVERSITI PUTRA MALAYSIA

***IDENTIFICATION AND DETERMINATION OF GENETIC DIVERSITY IN
SIMIAN MALARIA PARASITES AMONG WILD LONG-TAILED MONKEY
POPULATIONS FROM VARIOUS REGIONS OF PENINSULAR
MALAYSIA***

PARASTOO KHAJEAIAN

FBSB 2015 17



**IDENTIFICATION AND DETERMINATION OF GENETIC DIVERSITY
IN SIMIAN MALARIA PARASITES AMONG WILD LONG-TAILED
MONKEY POPULATIONS FROM VARIOUS REGIONS OF
PENINSULAR MALAYSIA**

By

PARASTOO KHAJEAIAN

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

March 2015

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 presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the Degree of Master of Science

**IDENTIFICATION AND DETERMINATION OF GENETIC DIVERSITY
IN SIMIAN MALARIA PARASITES AMONG WILD LONG-TAILED
MONKEY (*Macaca fascicularis*) POPULATIONS OF PENINSULAR
MALAYSIA**

By

PARASTOO KHAJEAIAN

March 2015

Chairman: Professor Tan Soon Guan / PhD

Faculty: Biotechnology and Biomolecular Sciences

Malaria still remains a major cause of mankind death globally in spite of a century of research. It is clear that, understanding and accurate measurement of malaria incidence as one of the most critical tropical diseases have significant role to control and prevent this lethal infection. Since the probability of *P. knowlesi* zoonotic transmission to humans was proven, the necessity of study on simian malaria parasites is increasingly obvious.

Simian malaria parasites are readily infectious to long-tailed (*Macaca fascicularis*) and pigtailed (*Macaca nemestrina*) monkeys. The chance of this pathogenic species switching to humans as their desired host is not ignorable due to the increase of human populations in recent years, as well as ecological alterations which are caused by pollution or deforestation. In fact macaques are preferred host for *Anopheles* mosquitoes but human may change this situation by alteration on the natural habitat of macaques and mosquitoes.

In this study, the distribution of five *Plasmodium* species namely: *P. knowlesi*, *P. inui*, *P. cynomolgi*, *P. fieldi* and *P. coatneyi* among the wild populations of *M. fascicularis* in six states in Peninsular Malaysia were determined using highly specific (nested-PCR) assays. The advantage of this method lies in its ability to detect very low numbers of parasites.

Monkey blood samples provided by the Department of Wild Life and National Parks Malaysia (PERHILITAN) were collected on Flinders Technical Association cards (FTA cards). FTA cards are strongly recommended for

collecting fluid samples for epidemiological studies particularly when sampling is being done in areas far from the main laboratory or during a long sampling trip. The prevalence of these five simian *Plasmodium* species was determined in 13 different locations of Peninsular Malaysia. Geographic distribution of the collected samples provided by PERHILITAN ranged from Northwest [Penang (island), Penang (mainland) and Jerejak Island], West (Selangor, Perak), Southwest (Negeri Sembilan), East (Pahang) and Northeastern (Kelantan) of Peninsular Malaysia. DNA was extracted from the blood spots on FTA Cards. All five simian *Plasmodium* species were successfully detected using nested PCR assay. Among the five species, *P. knowlesi* had the highest prevalence (34.3%), followed by *P. inui* (33.2%), *P. cynomolgi* (27.9%), *P. fieldi* (27.6%) and *P. coatneyi* (16.6%). Co-infections of macaques with multiple species of *Plasmodium* parasites were also observed. Kelantan had the highest prevalence rate among the states for all five simian malaria species. The incidence rate of three *Plasmodium* species which are *P. inui*, *P. fieldi* and *P. knowlesi* were higher than 50% among the samples obtained from this state.

Twenty positive DNA samples with single infection of *P. knowlesi* (12 samples) and *P. cynomolgi* (8 samples) as well as 20 uninfected monkey DNA samples were chosen to investigate the genetic diversity of these parasites using 26 different ISSR (inter simple sequence repeat) markers.

A total of 103 ISSR loci for infected samples and 95 for uninfected samples were generated. The analyses of the infected and uninfected samples using ISSR markers confirmed the efficiency of both the markers and the clustering methods. By these methods, the samples not only were separated according to their geographical distribution, but the samples were grouped into two distinct clusters according to the species of the malaria parasite.

Overall, this study shows the importance of research about malaria parasite species which are infectious to *M. fascicularis* and the necessity of preventive and control plans to decrease the chance of host-switch occurrence. This study also provides information for further investigations to design and develop diagnostic microsatellite markers for the macaques in the future.

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

**PENGENALPASTIAN DAN PENENTUAN KEPELBAGAIAN GENETIK
PARASIT MALARIA SIMIAN DI KALANGAN POPULASI MONYET
EKOR-PANJANG (*Macaca fascicularis*) LIAR DI SEMENANJUNG
MALAYSIA**

Oleh

PARASTOO KHAJEAIAN

Mac 2015

Pengerusi: Profesor Tan Soon Guan / PhD
Fakulti: Bioteknologi dan Sains Biomolekul

Malaria masih kekal sebagai punca utama kematian manusia di seluruh dunia walaupun penyelidikan telah dijalankan selama seabad. Adalah jelas bahawa, pemahaman dan pengukuran yang lebih tepat insiden penyakit malaria yang merupakan salah satu penyakit tropika yang paling kritikal mempunyai peranan signifikan untuk mengawal dan mencegah jangkitan maut ini. Oleh kerana kebarangkalian transmisi zoonotik *P. knowlesi* kepada manusia telah terbukti, keperluan kajian mengenaipasti parasit malaria monyet semakin ketara.

Parasit malaria monyet boleh berjangkit pada kera ekor-panjang (*Macaca fascicularis*) dan kera berekor pusaran (*Macacanemestrina*). Kebarangkalian spesies patogenik ini menukar kepada manusia sebagai hos tidak boleh diabaikan disebabkan peningkatan populasi manusia dalam tahun-tahun kebelakangan ini, dan juga perubahan ekologi yang disebabkan oleh pencemaran atau penebangan hutan. Sebenarnya, monyet adalah hos utama untuk nyamuk *Anopheles*, tetapi manusia boleh mengubah keadaan ini dengan perubahan pada habitat semula jadi monyet dan nyamuk.

Dalam kajian ini, distribusi lima spesies *Plasmodium* iaitu: *P. knowlesi*, *P. inui*, *P. cynomolgi*, *P. fieldi* dan *P. coatneyi* kalangan populasi liar *M. fascicularis* di enam negeri di Semenanjung Malaysia telah ditentukan dengan menggunakan ujian PCR-berperingkat spesifik. Kelebihan kaedah ini adalah pada keupayaannya untuk mengesan bilangan parasit yang amat rendah.

Sampel darah monyet yang disediakan oleh Jabatan Hidupan Liar dan Taman Negara Malaysia (PERHILITAN) telah dikumpulkan pada kertas tapis kad FTA. Kad FTA amat disyorkan untuk mengambil sampel cecair untuk kajian epidemiologi, terutamanya apabila persampelandilakukan di kawasan-kawasan jauh dari makmal atau semasa persampelan yang lama. Penularan lima spesies *Plasmodium* monyet ini telah ditentukan di 13 lokasi berlainan di Semenanjung Malaysia. Taburan geografi sampel yang disediakan oleh PERHILITAN adalah dari kawasan Barat Laut Pulau Pinang, Pulau Pinang (tanah besar), Pulau Jerejak, Barat (Selangor, Perak), Barat Daya (Negeri Sembilan), Timur (Pahang) dan Timur Laut (Kelantan) di Semenanjung Malaysia. DNA telah diekstrak daripada darah pada kad FTA. Kesemua lima spesies *Plasmodium* monyet telah berjaya dikesan menggunakan ujian PCR berperingkat. Antara lima spesies ini, *P. knowlesi* mempunyai kadar kejadian tertinggi (34.3%), diikuti oleh *P. inui* (33.2%), *P. cynomolgi* (27.9%), *P. fieldi* (27.6%) dan *P. coatneyi* (16.6%). Jangkitan pelbagai species parasit *Plasmodium* di dalam monyet juga diperhatikan. Kelantan mempunyai kadar kejadian tertinggi di kalangan negeri-negeri untuk semua lima spesies malaria monyet. Kadar insiden tiga spesies, iaitu *P. inui*, *P. fieldi* dan *P. knowlesi* adalah lebih tinggi daripada 50% di kalangan sampel yang dikumpul daripada negeri ini.

Dua puluh DNA sampel yang positif dengan jangkitan tunggal *P. knowlesi* (12 sampel) dan *P. cynomolgi* (8 sampel) serta 20 sampel DNA monyet yang tidak dijangkiti telah dipilih untuk menyiasat kepelbagaian genetik parasit ini dengan menggunakan 26 penanda ISSR (Inter Simple Sequence Repeat) yang berbeza. Sejumlah 103 ISSR lokus bagi sampel yang dijangkiti dan 95 untuk sampel yang tidak dijangkiti telah dijana. Analisis sampel yang dijangkiti dan tidak dijangkiti menggunakan penanda ISSR telah mengesahkan kecekapan kedua-dua penanda dan kaedah kelompok. Dengan kaedah ini, sampel bukan sahaja telah diasingkan mengikut taburan geografi mereka, tetapi sampel-sampel juga dikumpulkan ke dalam dua kelompok yang nyata ssssssmengikat spesies parasit malaria.

Secara keseluruhannya, kajian ini menunjukkan betapa pentingnya kajian spesies parasit malaria yang berupaya untuk menjangkiti *M. fascicularis* dan keperluan pencegahan dan rancangan kawalan untuk mengurangkan peluang penukaran hos berlaku. Kajian ini juga menyediakan maklumat untuk siasatan lanjut untuk mereka dan membangunkan penanda mikrosatelit diagnostik untuk monyet pada masa hadapan.



ACKNOWLEDGEMENTS

First and foremost, my gratitude goes to my truly kind and devoted supervisor Professor Dr. Soon-Guan Tan. I would never conduct this research without his continuing guidance and effective suggestions. His ever-ethical devotion to science became my inspiration to research style. I especially want to extend my heartfelt thanks and deepest gratitude to my dear co-supervisor Dr. Christina Yong who was incredibly efficient, supportive and excellent in guiding me through this novel experience. My grateful appreciation is also due to my co-supervisors Associated Professor Dr. Noorjahan Banu Mohammed Alitheen, Dr. Reuben Sharma and Mr. Jeffrine Rovie Ryan Japning who were always supportive and never withheld of providing whatever I needed.

I acknowledge The Department of Wildlife and National Parks. A special appreciation goes to Mr. Frankie Anak Thomas Sitam who assisted me through the initial steps of this study. I am certainly grateful and indebted to Dr. Alireza Valdiani for his valuable and effectual helps and ideas until the last minute of my study.

I wish to express my sincere appreciation to my close friends Maryam Roshani, Sareh Seyedi and Sonia Nikzad for their support and kind wishes.

My profound and warmest thanks and love to my husband Mohammad and my beloved sons Reza and Ilia for their love, patience, and encouragement and to my father for his constant support, love and prayers throughout my study. Last but certainly not least, I wish to express my sincere appreciation to all those who were not mentioned here that helped me to ensure the completion of my research.

I certify that a Thesis Examination Committee has met on 24 March 2015 to conduct the final examination of Parastoo Khajeaian on her thesis entitled "Identification and Determination of Genetic Diversity in Simian Malaria Parasites among Wild Long-Tailed Monkey Populations from Various Regions of 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.

Members of the Thesis Examination Committee were as follows:

Parameswari a/p Namasivayam, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Cheah Yoke Kqueen, PhD

Professor

Faculty of Medicine and Health Sciences

Universiti Putra Malaysia

(Internal Examiner)

Patimah binti Ismail, PhD

Professor

Faculty of Medicine and Health Sciences

Universiti Putra Malaysia

(Internal Examiner)

Wan Kiew Lian, PhD

Professor

Faculty of Science and Technology

Universiti Kebangsaan Malaysia

(External Examiner)



ZULKARNAIN ZAINAL, PhD

Professor and Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

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

Tan Soon Guan, PhD

Professor
Faculty of Biotechnology
Universiti Putra Malaysia
(Chairman)

Christina Yong, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Noorjahan Banu Mohammed Alitheen, PhD

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

Reuben Sunil Kumar Sharma, Senior lecturer

Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Jeffrine Rovie Ryan Japning

Department of Wildlife and National Parks
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
- the thesis has not been submitted previously or concurrently for any other degree at any 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 owned from supervisor and 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.: Parastoo Khajeaian GS30640

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) were adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
1.1 General Background	2
1.2 Hypothesis	3
1.3 Main objective	3
1.4 Specific objective	3
2 LITERATURE REVIEW	4
2.1 Cynomolgus monkey	4
2.1.1 Classification of monkey	4
2.1.2 Morphological and native characteristics	5
2.2 Malaria	7
2.2.1 Malaria in Malaysia	9
2.2.2 Classification of parasite	10
2.2.3 Life cycle of malaria parasite	10
2.2.4 Non-human primate malarias	12
2.2.5 Possibility of simian malaria occurring in human host	13
2.2.6 History of natural infection of <i>P. knowlesi</i> in human host	14
2.3 Methods in detection and identification of simian malaria parasites	15
2.3.1 Microscopic observation	15
2.3.2 Polymerase Chain Reaction (PCR) assays	16
2.3.3 Screening of simian malaria parasites	16
2.3.3.1 Filter papers	16
2.3.3.2 Nested PCR assay	18
2.3.3.4 Direct nested PCR	18
2.4 Genetic diversity	18
2.4.1 Characteristics of Microsatellites	19
2.4.1.1 Microsatellites Isolation	19
2.4.1.2 Cross- species amplification	20
2.4.1.3 Microsatellites in Plasmodium	20

2.4.2 Inter-simple sequence repeats (ISSRs)	20
	22
3 MATERIALS AND METHODS	22
3.1 Populations and animal sampling	23
3.2 DNA Extraction	23
3.2.1 Filter papers (FTA cards)	23
3.2.1.1 Preparation protocol of filter papers	23
3.2.1.2 Purification of DNA on filter papers	24
3.2.1.3 DNA extraction from filter papers	25
3.2.2 Whole blood	26
3.2.2.1 DNA Extraction from whole blood samples	26
3.3 Screening the population using Nested- PCR method	26
3.3.1 Plasmodium genus-specific nested PCR condition	26
3.3.2 Plasmodium species-specific nested PCR conditions	27
3.3.3 Control tubes for PCR	28
3.3.4 Agarose gel electrophoresis (1.5 %)	29
3.3.5 Gel purification of PCR product	29
3.3.6 Data Analysis	30
3.4 Simple sequence repeats (SSRs)	30
3.4.1 Optimization of PCR Conditions	33
3.4.2 Polyacrylamide gel electrophoresis (PAGE) 8 %	34
3.4.3 Isolation of DNA fragments	34
3.4.4 Data Analysis	34
3.5 Inter-simple sequence repeats (ISSRs)	35
3.5.1 DNA amplification	35
3.5.2 Data analysis	35
4 RESULTS	37
4.1 DNA extraction	37
4.2 Screening of the wild monkey populations for Plasmodium parasites	37
4.3 Comparison of the direct and standard nested PCR	44
4.4 Genetic diversity using inter simple sequence repeat (ISSRs)	45
4.4.1 ISSR polymerase chain reaction (PCR) analysis of the infected macaque population	45
4.4.2 ISSR-based cluster analysis in the infected macaque populations	49
4.4.3 ISSR polymerase chain reaction (PCR) analysis of the uninfected macaque population	51
4.4.4 ISSR-based cluster analysis in non- infected macaque population	55

5 DISCUSSION	57
5.1 Prevalence of malaria disease among the wild monkey populations	57
5.2FTA papers and direct nested PCR method to detect malaria parasites	59
5.3 Genetic diversity using inter simple sequence repeats (ISSR)	59
5.3.1 ISSR based genetic diversity of the infected and uninfected <i>M. fascicularis</i> samples	60
5.3.2 Comparison of the nucleotides frequencies in Plasmodium and Macaque genomes	61
6 CONCLUSIONS	62
REFERENCES	64
APPENDICES	80
BIODATA OF STUDENT	128
LIST OF PUBLICATIONS	129

LIST OF TABLES

Table		Page
2.1	Scientific classification of monkey (<i>M. fascicularis</i>)	4
2.2	The Scientific classification of malaria Parasite	10
2.3	List of non-human primate <i>Plasmodium</i> species	13
3.1	Sampling locations and sample sizes of <i>M. fascicularis</i>	23
3.2	Sequences and annealing temperatures of Genus- specific and species-specific PCR primers used in nested PCR	28
3.3	Microsatellite loci used in this study	31
3.4	Cycling parameters for PCR	33
4.1	Summary of malaria infections in macaques from different age groups and genders sampled in this study	39
4.2	Breakdown of various combinations of <i>plasmodium</i> species observed in infected macaques	41
4.3	Percentage of infection for each species in any of the six states studied	43
4.4	Comparison of malaria diagnoses by direct and standard nested PCR.	44
4.5	Polymorphic loci generated with ISSR primers in infected macaque population	46
4.6	Polymorphic loci generated with ISSR primers in uninfected macaque populations	50

LIST OF FIGURES

Figure		Page
2.1	A long-tailed macaque found in Fraser's Hill	5
2.2	<i>M. fascicularis</i> geographical distribution range	7
2.3	Latest malaria reported cases globally in 2012	8
2.4	Life cycle of malaria parasite involves invertebrate and intermediate vertebrate hosts	11
2.5	Filter papers with monkey blood applied to it	17
3.1	2.0 mm diameter Harris micro puncher	24
4.1	Electrophoretic characterization of genomic DNA isolated from A) FTA cards and B) blood	37
4.2	Gel electrophoresis of five malaria parasite species PCR product using 1.5% (W/V) agarose gel	38
4.3	Comparison of the prevalence of five <i>plasmodium</i> species screened in this study	40
4.4	Prevalence rate of <i>plasmodium</i> parasite in different states of Peninsular Malaysia	42
4.5	Amplification of genomic DNA of Some infected individuals with ISSR primers UBC808 and UBC811	48
4.6	ISSR-based dendrogram of the 20 infected monkeys generated by UPGMA clustering method and Jaccard's similarity matrices	50
4.7	Amplification of genomic DNA of Some uninfected individuals with ISSR primers UBC809 and UBC815	54
4.8	ISSR-based dendrogram of the 20 uninfected monkeys generated by UPGMA clustering method and Jaccard's similarity matrices	56

LIST OF ABBREVIATIONS

µL	Microliter
%	Percentage
°C	Centigrade Celsius
1X	One time
A	Adenine
AIDS	Acquired Immuno Defficiency Syndrom
AFLP	Amplified Fragment Length Polymerase
APS	Ammonium Persulfate
B.C	Before Christ
bp	Base pairs
BLAST	Basic Local Alignment Search Tool
C	Cytosine
CDC	Centre for Disease Control
CQ	chloroquine
ddH ₂ O	deionised distilled water
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EtBr	Ethidium bromide
FTA	Flinders Technical Association
G	Guanine
G	Gram
ISSR	Inter Simple Sequence Repeat
Km	Kilometer
Mg	Milligram
MgCl ₂	Magnesium chloride
min	Minute
ml	Milliliter

mM	Milimolar
mm	Milimeter
MS	Microsatellites
ng	Nanogram
NCBI	National Center for Biotechnology Information
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	ribonucleic acid
rpm	rotation per minute
sec	Second
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
SSR	Simple Sequence Repeat
SSU rRNA	small sub-unit ribosomal ribonucleic acid
T	Thymine
TBE	Tris-Borate-EDTA
U/ μ L	Unite per microliter
UPGMA	Unweighted Pair Group Method with Arithmetic mean
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Volt
WHO	World Health Organization
W/V	Weight per volume
X	Times

CHAPTER 1

INTRODUCTION

1.1 General Background

In the midst of the last century it was believed to be the end of contagious diseases as a result of the development of several different drugs and vaccines (Fauci, 2001). Unfortunately this outlook has been proven wrong due to a variety of reasons, such as the failure to understand the evolutionary potential of parasites (generally described as including viruses, bacteria and eukaryotic micro- and macro parasites). For instance, visiting hospitals nowadays increases the risk of the acquisition of antibiotic-resistant infections (Peleg and Hooper, 2010). Recently, a totally drug-resistant tuberculosis has been recognized in India (Udwadia *et al.*, 2012), and the emergence of resistance to the latest frontline anti malarial drugs has been proven in many areas (Cheeseman *et al.*, 2012). Although the significant role of drugs and vaccines in the control of numerous infectious diseases are obvious, eradication has only succeeded in a few cases. It is concerning and quite surprising that the evolutionary considerations are not better integrated into biomedicine and control policies, given that infectious diseases are responsible for substantial morbidity and mortality of humans, wildlife, livestock and agricultural crops (WHO, 2013).

The fast evolution of parasites makes them important to understand from the applied perspectives; which also makes them very interesting and helpful models for studying basic evolutionary processes. Several factors which make parasites capable of fast evolution: short generation times, large population sizes, considerable genetic and phenotypic variation that may be generated through sexual reproduction and variable mutation rates (Poulin, 2007; Stearns and Koella, 2007). Monitoring studies of malaria parasites among the long-tailed macaques (*Macaca fascicularis*) in Malaysia revealed that this species have the maximum prevalence of malaria compared to the other macaque species (Eyles, 1963). Long-tailed macaques (*M. fascicularis*) (Raffles, 1821) are generally distributed in mainland and insular Southeast Asia from 21°N to 10°S latitude and from 92°E to 126°E longitude, covering the Philippines, Indonesia, Malaysia, Cambodia, Mauritius, Vietnam, Thailand, Myanmar, Singapore and Timor-Leste (Groves, 2001). After human and rhesus macaques, the long-tailed macaques have the third most wide-ranging geographical spread among primates (Fooden, 1995).

They used to live in the forests and near human habitations. Previous studies have found that this species of macaque is the natural host for human *Plasmodium knowlesi* infection (Jeslyn *et al.*, 2011). It is certainly necessary to determine the prevalence of simian malaria parasites in local macaques so that the risk of potential malaria hooknoses may be recognized. It is known that *P. knowlesi* infection could be severe and fatal in humans (Cox-Singh *et al.*, 2010; Galinski and Barnwell, 2009) while some other species of simian malaria parasites such as *Plasmodium cynomolgi* and *Plasmodium inui*, have now been proven to be infectious to humans too (Galinski and Barnwell, 2009).

The record of the locally acquired *P. knowlesi* cases and the subsequent detection of *P. knowlesi* parasites in samples of local wild long-tailed macaques show a potential risk of zoonotic transmission of malaria infection in Malaysia (Tan *et al.*, 2008). However, there is a need to screen for simian malaria parasites in more substantial populations of macaques, as only a small sample of macaques were tested formerly for the presence of this parasite. For a much better understanding on the prevalence of malaria disease in local macaques, it is preferable to collect samples from various geographical locations. This is to enabling the evaluation of the risk of zoonotic transmission of simian malaria parasites to the human population from the long tail monkey. Recent achievements in molecular methods, especially the polymerase chain reaction (PCR) enabled scientists to genotype malaria parasites directly from the patient's blood samples without any prior *in vitro* culture needed. Molecular markers are efficient tools for genetic analyses, taxonomic classification, studying the phylogenetic relationship, as well as prognostic studies in different taxa (Valdiani *et al.*, 2014). The inter-simple sequence repeats (ISSRs) can be useful and beneficial in this era.

1.2 Hypothesis

The long-tailed macaques are the biggest population of non-human primates in Malaysia. In addition to *P. knowlesi*, this species of macaques is also harboring *P. cynomolgi*, *P. inui*, *P. fieldi* and *P. coatneyi* (Coatney *et al.*, 1971). However to-date, there has been no reports on the prevalence of simian malaria in peninsular Malaysia. Macaques as the natural hosts of *P. knowlesi* inhabit in various public nature parks near to human population. Since most of the surveillance studies regard natural incidence of simian malaria had been conducted in Kapit Division of Sarawak, East Malaysian on the Borneo Island (Lee *et al.*, 2011), This study will provide evidence to show the presence and ongoing transmission of malaria parasites among local macaques and answer the question if there is a need to be alarmed for the risk of the general population acquiring zoonotic malaria. In this thesis study of evolutionary

biology with tools from molecular biology will allow us to study macaque malaria parasites at both evolutionary and ecological aspects.

1.3 Main objective

The general objective of this project was to determine the prevalence of simian malaria parasites in the long-tailed macaques of Peninsular Malaysia.

1.4 Specific objective

2. To determine the incidence of five malaria parasites namely *P. knowlesi*, *P. inui*, *P. cynomolgi*, *P. fieldi* and *P. coatneyi* in samples of long-tailed macaque (*M. fascicularis*) from Peninsular Malaysia.
3. To assess the genetic diversity using inter simple sequence repeat (ISSR)
4. To compare the sensitivity and accuracy of direct nested - PCR method with standard nested - PCR method.

REFERENCES

- Ameri, M. (2010). Laboratory diagnosis of malaria in nonhuman primates. *Veterinary Clinical Pathology*, 39(1), 5-19.
- Anderson, T. J., Haubold, Williams, J. T., Estrada-Franco, J. G., Richardson, L., Mollinedo, R., *et al.* (2000). Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Molecular Biology and Evolution*, 17(10), 1467-1482.
- Anderson, T. J., Su, X. Z., Roddam, A., and Day, K. P. (2000). Complex mutations in a high proportion of microsatellite loci from the protozoan parasite *Plasmodium falciparum*. *Molecular Ecology*, 9(10), 1599-1608.
- Awise, J. C. (2010). Perspective: conservation genetics enters the genomics era. *Conservation Genetics*, 11(2), 665-669.
- Awise, J. C., Jones, A. G., Walker, D., and DeWoody, J. A. (2002). Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. *Annual Review of Genetics*, 36(1), 19-45.
- Baird, J. K. (2009). Malaria zoonoses. *Travel Medicine and Infectious Disease*, 7(5), 269-277.
- Barr, C. S., Newman, T. K., Becker, M. L., Parker, C. C., Champoux, M., Lesch, K., *et al.* (2003). The utility of the non-human primate model for studying gene by environment interactions in behavioral research. *Genes, Brain and Behavior*, 2(6), 336-340.
- Becker, J., and Heun, M. (1995). Mapping of digested and undigested random amplified microsatellite polymorphisms in barley. *Genome*, 38(5), 991-998.
- Blair, M., Panaud, O., and McCouch, S. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 98(5), 780-792.
- Blancher, A., Bonhomme, M., Crouau-Roy, B., Terao, K., Kitano, T., and Saitou, N. (2008). Mitochondrial DNA sequence phylogeny of 4 populations of the widely distributed cynomolgus macaque (*Macaca fascicularis*). *Journal of Heredity*, 99(3), 254-264.

- Bornet, B., and Branchard, M. (2004). Use of ISSR fingerprints to detect microsatellites and genetic diversity in several related *Brassica taxa* and *Arabidopsis thaliana*. *Hereditas*, 140(3), 245-247.
- Bottius, E., Guanzirolli, A., Trape, J.-F., Rogier, C., Konate, L., and Druilhe, P. (1996). Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90(1), 15-19.
- Brito, C. F. A. d., and Ferreira, M. U. (2011). Molecular markers and genetic diversity of *Plasmodium vivax*. *Memórias do Instituto Oswaldo Cruz*, 106, 12-26.
- Bronner, U., Divis, P. C., Färnert, A., and Singh, B. (2009). Swedish traveller with *Plasmodium knowlesi* malaria after visiting Malaysian Borneo. *Malaria Journal*, 8(1), 15.
- Capuano, S. V., Croix, D. A., Pawar, S., Zinovik, A., Myers, A., Lin, P. L., et al. (2003). Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. *Infection and Immunity*, 71(10), 5831-5844.
- Carter, R., and Voller, A. (1973). Enzyme variants in primate malaria parasites. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 67(1), 14-15.
- Chakraborty, D., Ramakrishnan, U., Panor, J., Mishra, C., and Sinha, A. (2007). Phylogenetic relationships and morphometric affinities of the Arunachal macaque *Macaca munzala*, a newly described primate from Arunachal Pradesh, northeastern India. *Molecular Phylogenetics and Evolution*, 44(2), 838-849.
- Chakraborty, D., and Smith, D. G. (2013). Anthropogenic influences on macaque populations and their genetic consequences *The Macaque Connection* (209-224): Springer.
- Chang, M., Hii, J., Buttner, P., and Mansoor, F. (1997). Changes in abundance and behaviour of vector mosquitoes induced by land use during the development of an oil palm plantation in Sarawak. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 91(4), 382-386.

- Charters, Y., and Wilkinson, M. (2000). The use of self-pollinated progenies as 'in-groups' for the genetic characterization of cocoa germplasm. *Theoretical and Applied Genetics*, 100(1), 160-166.
- Cheeseman, I. H., Miller, B. A., Nair, S., Nkhoma, S., Tan, A., Tan, J. C., *et al.* (2012). A major genome region underlying artemisinin resistance in malaria. *Science*, 336(6077), 79-82.
- Chin, W., Contacos, P. G., Coatney, G. R., and Kimball, H. R. (1965). A naturally acquired quotidian-type malaria in man transferable to monkeys. *Science*, 149, 865.
- Chin, W., Contacos, P. G., Collins, W. E., Jeter, M. H., and Alpert, E. (1968). Experimental mosquito-transmission of *Plasmodium knowlesi* to man and monkey. *The American Journal of Tropical Medicine and Hygiene*, 17(3), 355-358.
- Chu, J.-H., Lin, Y.-S., and Wu, H.-Y. (2007). Evolution and dispersal of three closely related macaque species, *Macaca mulatta*, *M. cyclopis*, and *M. fuscata*, in the eastern Asia. *Molecular Phylogenetics and Evolution*, 43(2), 418-429.
- Cnops, L., Jacobs, J., and Van Esbroeck, M. (2011). Validation of a four-primer real-time PCR as a diagnostic tool for single and mixed *Plasmodium* infections. *Clinical Microbiology and Infection*, 17(7), 1101-1107.
- Coatney. (1963). Simian malaria: its importance to world-wide eradication of malaria. *JAMA*, 184(11), 876-877.
- Coatney, Elder, H. A., Contacos, P. G., Getz, M. E., Greenland, R., Rossan, R. N., *et al.* (1961). Transmission of the M strain of *Plasmodium cynomolgi* to man. *The American Journal of Tropical Medicine and Hygiene*, 10, 673.
- Coatney, G. R., Collins, W. E., Warren, M., and Contacos, P. G. (1971). The primate malarias. *The Primate Malarias*, 366.
- Cogswell. (1992). The hypnozoite and relapse in primate malaria. *Clinical Microbiology Reviews*, 5(1), 26-35.
- Cogswell. (2000). Malaria and piroplasms of non-human primates. *International Veterinary Information Service, Ithaca, NY (www. ivis. org)*, 2000, A0304.

- Coleman, R. E., Sattabongkot, J., Promstaporm, S., Maneechai, N., Tippayachai, B., Kengluetcha, A., *et al.* (2006). Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. *Malaria Journal*, 5(1), 121.
- Collins, W. E. (2012). *Plasmodium knowlesi*: A Malaria parasite of monkeys and humans. *Annual Review of Entomology*, 57, 107-121.
- Collins, W. E., and Contacos, P. G. (1971). Observations on the relapse activity of *Plasmodium fieldi* in the rhesus monkey. *The Journal of Parasitology*, 29-32.
- Collins, W. E., Warren, M., Sullivan, J. S., Galland, G. G., Nace, D., Williams, A., *et al.* (2005). Studies on two strains of *Plasmodium cynomolgi* in New World and Old World monkeys and mosquitoes. *Journal of Parasitology*, 91(2), 280-283.
- Contacos, P. G., Elder, H. A., Coatney, G. R., and Genther, C. (1962). Man to man transfer of two strains of *Plasmodium cynomolgi* by mosquito bite. *The American Journal of Tropical Medicine and Hygiene*, 11(2), 186-193.
- Cox-Singh, J., Davis, T. M., Lee, K.-S., Shamsul, S. S., Matusop, A., Ratnam, S., *et al.* (2008). *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases*, 46(2), 165-171.
- Cox-Singh, J., Hiu, J., Lucas, S. B., Divis, P. C., Zulkarnaen, M., Chandran, P., *et al.* (2010). Severe malaria-a case of fatal *Plasmodium knowlesi* infection with post-mortem findings. *Malaria Journal*, 9(10).
- Cox-Singh, J., Mahayet, S., Abdullah, M. S., and Singh, B. (1997). Increased sensitivity of malaria detection by nested polymerase chain reaction using simple sampling and DNA extraction. *International Journal for Parasitology*, 27(12), 1575-1577.
- Daniels, C. (1908). Animal parasites in man and some of the lower animals in Malaya. *Studies of the Institute Medical Research EMS*, 3, 1-13.
- Dieringer, D., and Schlötterer, C. (2003). Two distinct modes of microsatellite mutation processes: evidence from the complete genomic sequences of nine species. *Genome Research*, 13(10), 2242-2251.
- Ellegren, H. (2000). Microsatellite mutations in the germline implications for evolutionary inference. *Trends in Genetics*, 16(12), 551-558.

- Emborg, M. E. (2007). Nonhuman primate models of Parkinson's disease. *ILAR Journal*, 48(4), 339-355.
- Ender, A., Schwenk, K., Städler, T., Streit, B., and Schierwater, B. (1996). RAPD identification of microsatellites in *Daphnia*. *Molecular Ecology*, 5(3), 437-441.
- Ennis, J., Teal, A., Habura, A., Madison-Antenucci, S., Keithly, J., Arguin, P., et al. (2009). Simian malaria in a US traveler-New York, 2008. *Morbidity and Mortality Weekly Report*, 58(9), 229-232.
- Eudey, A. A. (2008). The crab-eating macaque (*Macaca fascicularis*): Widespread and rapidly declining. *Primate Conservation*, 23(1), 129-132.
- Eyles. (1963). The species of simian malaria: Taxonomy, morphology, life cycle, and geographical distribution of the monkey species. *The Journal of Parasitology*, 49, 866.
- Fauci, A. S. (2001). Infectious diseases: considerations for the 21st century. *Clinical Infectious Diseases*, 32(5), 675-685.
- Figtree, M., Lee, R., Bain, L., Kennedy, T., Mackertich, S., Urban, M., et al. (2010). *Plasmodium knowlesi* in human, Indonesian Borneo. *Emerging Infectious Diseases*, 16(4).
- Fong, Y. L., Cadigan, F. C., and Robert Coatney, G. (1971). A presumptive case of naturally occurring *Plasmodium knowlesi* malaria in man in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 65(6), 839-840.
- Fooden, J. (1976). Provisional classification and key to living species of macaques (Primates: *Macaca*). *Folia Primatologica*, 25(2-3), 225-236.
- Fooden, J. (1994). Malaria in macaques. *International Journal of Primatology*, 15(4), 573-596.
- Fooden, J. (1995). Systematic review of Southeast Asian longtail macaques, *Macaca fascicularis* (Raffles, 1821). *Fieldiana Zoology.*, 81, 1-206.
- Fuehrer, H.-P., Fally, M. A., Habler, V. E., Starzengruber, P., Swoboda, P., and Noedl, H. (2011). Novel nested direct PCR technique for malaria diagnosis using filter paper samples. *Journal of Clinical Microbiology*, 49(4), 1628-1630.

- Galinski, M. R., and Barnwell, J. W. (2009). Monkey malaria kills four humans. *Trends in Parasitology*, 25(5), 200-204.
- Garnham, P. C. C. (1966). Malaria parasites and other Haemosporidia. *Malaria Parasites and Other Haemosporidia.*, 1114-1119.
- Goode, T., Ho, W.-Z., O'Connor, T., Busted, S., Douglas, S. D., Shanahan, F., et al. (2002). Nested RT-PCR. *RT-PCR Protocols* (65-79): Springer.
- Groves, C. P. (2001). Primate taxonomy. citeulike.org
- Gupta, M., Chyi, Y.-S., Romero-Severson, J., and Owen, J. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, 89(7-8), 998-1006.
- Gysin, J. (1998). Animal models: primates. *Malaria: Parasite biology, Pathogenesis, and Protection*, 419-441.
- Hamada, Y., Watanabe, T., Takenaka, O., Suryobroto, B., and Kawamoto, Y. (1988). Morphological studies on the Sulawesi macaques I: phyletic analysis of body color. *Primates*, 29(1), 65-80.
- Harrison, T., Krigbaum, J., and Manser, J. (2006). Primate biogeography and ecology on the Sunda Shelf islands: a paleontological and zooarchaeological perspective *Primate Biogeography*(331-372): Springer.
- Hasan, M. K., Aziz, M. A., Alam, S. R., Kawamoto, Y., Engel, L. J.-., Kyes, R. C., et al. (2013). Distribution of rhesus macaques (*Macaca mulatta*) in Bangladesh: inter-population variation in group size and composition. *Primate Conservation*, 26(1), 125-132.
- Held, J. R., Contacos, P. G., and Coatney, G. R. (1967). Studies of the exoerythrocytic stages of simian malaria. *Plasmodium fieldi*. *The Journal of Parasitology*, 225-232.
- Hii, J. L., Kan, S., Vun, Y., Chin, K., Lye, M., Mak, J., et al. (1985). *Anopheles flavirostris* incriminated as a vector of malaria and *Bancroftian filariasis* in Banggi Island, Sabah, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79(5), 677-680.

- Hoosen, A., and Shaw, M. (2011). *Plasmodium knowlesi* in a traveller returning to New Zealand. *Travel Medicine and Infectious Disease*, 9(3), 144-148.
- Hu, J., Nakatani, M., Lalusin, A. G., Kuranouchi, T., and Fujimura, T. (2003). Genetic analysis of sweetpotato and wild relatives using inter-simple sequence repeats (ISSRs). *Breeding Science*, 53(4), 297-304.
- Hwang, J., Jaroensuk, J., Leimanis, M. L., Russell, B., McGready, R., Day, N., *et al.* (2012). Long-term storage limits PCR-based analyses of malaria parasites in archival dried blood spots. *Malaria Journal*, 11, 339.
- Jeslyn, W. P. S., Huat, T. C., Vernon, L., Irene, L. M. Z., Sung, L. K., Jarrod, L. P., *et al.* (2011). Molecular epidemiological investigation of *Plasmodium knowlesi* in humans and macaques in Singapore. *Vector-Borne and Zoonotic Diseases*, 11(2), 131-135.
- Jiram, A. I., Vythilingam, I., NoorAzian, Y. M., Yusof, Y. M., Azahari, A. H., and Fong, M.-Y. (2012). Entomologic investigation of *Plasmodium knowlesi* vectors in Kuala Lipis, Pahang, Malaysia. *Malaria Journal*, 11(1), 213.
- Jongwutiwes, S., Putaporntip, C., Iwasaki, T., Sata, T., and Kanbara, H. (2004). Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. *Emerging Infectious Diseases*, 10(12).
- Joshi, S., Gupta, V., Aggarwal, R., Ranjekar, P., and Brar, D. (2000). Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics*, 100(8), 1311-1320.
- Kain, K. C., and Keystone, J. S. (1998). Malaria in travelers: epidemiology, disease, and prevention. *Infectious Disease Clinics of North America*, 12(2), 267-284.
- Karunaweera, N., Ferreira, M., Hartl, D., and Wirth, D. (2007). Fourteen polymorphic microsatellite DNA markers for the human malaria parasite *Plasmodium vivax*. *Molecular Ecology Notes*, 7(1), 172-175.
- Kuiken, T., Rimmelzwaan, G., Van Amerongen, G., and Osterhaus, A. (2003). Pathology of human influenza A (H5N1) virus infection in cynomolgus macaques (*Macaca fascicularis*). *Veterinary Pathology Online*, 40(3), 304-310.

- Küpper, C., Burke, T., Székely, T., and Dawson, D. A. (2008). Enhanced cross-species utility of conserved microsatellite markers in shorebirds. *BMC Genomics*, 9(1), 502.
- Leclerc, M., Durand, P., Gauthier, C., Patot, S., Billotte, N., Menegon, M., *et al.* (2004). Meager genetic variability of the human malaria agent *Plasmodium vivax*. *Proceedings of the National Academy of Sciences of the United States of America*, 101(40), 14455-14460.
- Lee, K.-S., Divis, P. C., Zakaria, S. K., Matusop, A., Julin, R. A., Conway, D. J., *et al.* (2011). *Plasmodium knowlesi*: reservoir hosts and tracking the emergence in humans and macaques. *PLoS Pathogens*, 7(4), e1002015.
- Leuchte, N., Berry, N., Köhler, B., Almond, N., LeGrand, R., Thorstensson, R., *et al.* (2004). MhcDRB-sequences from cynomolgus macaques (*Macaca fascicularis*) of different origin. *Tissue Antigens*, 63(6), 529-537.
- Levine, N. D. (1988). *The protozoan phylum Apicomplexa. Volume I. Volume II*: CRC Press, Inc.
- Lewontin, R. C. (1995). The apportionment of human diversity *Evolutionary Biology* (pp. 381-398): Springer.
- Li, Q.-Q., and Zhang, Y.-P. (2005). Phylogenetic relationships of the macaques (*Cercopithecidae: Macaca*), inferred from mitochondrial DNA sequences. *Biochemical Genetics*, 43(7-8), 375-386.
- Liu, W., Li, Y., Learn, G. H., Rudicell, R. S., Robertson, J. D., Keele, B. F., *et al.* (2010). Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature*, 467(7314), 420-425.
- Longini, I. M., Nizam, A., Xu, S., Ungchusak, K., Hanshaworakul, W., Cummings, D. A., *et al.* (2005). Containing pandemic influenza at the source. *Science*, 309(5737), 1083-1087.
- Luchavez, J., Espino, F., Curameng, P., Espina, R., Bell, D., Chiodini, P., *et al.* (2008). Human infections with *Plasmodium knowlesi*, the Philippines. *Emerging Infectious Diseases*, 14(5), 811.
- Maresso, K., and Broeckel, U. (2008). Genotyping platforms for mass-throughput genotyping with SNPs, including human genome-wide scans. *Advances in Genetics*, 60, 107-139.

- McClure, M. C., McKay, S. D., Schnabel, R. D., and Taylor, J. F. (2009). Assessment of DNA extracted from FTA® cards for use on the Illumina iSelect BeadChip. *BMC Research Notes*, 2(1), 107.
- Metzgar, D., Bytof, J., and Wills, C. (2000). Selection against frameshift mutations limits microsatellite expansion in coding DNA. *Genome Research*, 10(1), 72-80.
- Miles, M., and Saul, D. (2007). Improved elution of DNA from Whatman FTA® cards using prepGEM®/forensicGEM® Storage Card extraction kits.
- Moore, S., Sargeant, L., King, T., Mattick, J., Georges, M., and Hetzel, D. (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics*, 10(3), 654-660.
- Mu, J., Joy, D. A., Duan, J., Huang, Y., Carlton, J., Walker, J., *et al.* (2005). Host switch leads to emergence of *Plasmodium vivax* malaria in humans. *Molecular Biology and Evolution*, 22(8), 1686-1693.
- Muentener, P., Schlagenhauf, P., and Steffen, R. (1999). Imported malaria (1985-95): trends and perspectives. *Bulletin-World Health Organization*, 77, 560-566.
- Muhsin, A.-M. A. A., Mackinnon, M. J., Ali, E., Nassir, E.-K. A., Suleiman, S., Ahmed, S., *et al.* (2004). Evolution of drug-resistance genes in *Plasmodium falciparum* in an area of seasonal malaria transmission in Eastern Sudan. *Journal of Infectious Diseases*, 189(7), 1239-1244.
- Neff, B. D., and Gross, M. R. (2001). Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. *Evolution*, 55(9), 1717-1733.
- Ng, O. T., Ooi, E. E., Lee, C. C., Lee, P. J., Ng, L. C., Pei, S. W., *et al.* (2008). Naturally acquired human *Plasmodium knowlesi* infection, Singapore. *Emerging Infectious Diseases*, 14(5), 814.
- Noedl, H., Socheat, D., and Satimai, W. (2009). Artemisinin-resistant malaria in Asia. *New England Journal of Medicine*, 361(5), 540-541.
- Noor, M. A., and Feder, J. L. (2006). Speciation genetics: evolving approaches. *Nature Reviews Genetics*, 7(11), 851-861.

- Oliveira, E. J., Pádua, J. G., Zucchi, M. I., Vencovsky, R., and Vieira, M. L. C. (2006). Origin, evolution and genome distribution of microsatellites. *Genetics and Molecular Biology*, 29(2), 294-307.
- Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin of the World Health Organization*, 66(5), 621.
- Peleg, A. Y., and Hooper, D. C. (2010). Hospital-acquired infections due to gram-negative bacteria. *New England Journal of Medicine*, 362(19), 1804-1813.
- Perwitasari-Farajallah, D., Kyes, R. C., and Iskandar, E. (2010). Microsatellite DNA polymorphisms for colony management of longtailed macaques (*Macaca fascicularis*) population on the Tinjil Island. *Biodiversitas*, 11, 55-58.
- Poulin, R. (2007). Are there general laws in parasite ecology? *Parasitology*, 134(06), 763-776.
- Primmer, Møller, A., and Ellegren, H. (1996). A wide-range survey of cross-species microsatellite amplification in birds. *Molecular Ecology*, 5(3), 365-378.
- Primmer, N Painter, J., T Koskinen, M., U Palo, J., and Merilä, J. (2005). Factors affecting avian cross-species microsatellite amplification. *Journal of Avian Biology*, 36(4), 348-360.
- Putaporntip, C., Hongsriruang, T., Seethamchai, S., Kobasa, T., Limkittikul, K., Cui, L., et al. (2009). Differential prevalence of *Plasmodium* infections and cryptic *Plasmodium knowlesi* malaria in humans in Thailand. *Journal of Infectious Diseases*, 199(8), 1143-1150.
- Qian, W., Ge, S., and Hong, D.-Y. (2001). Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics*, 102(2-3), 440-449.
- Raffles, T. S. (1821). XVII. Descriptive Catalogue of a Zoological Collection, made on account of the Honourable East India Company, in the Island of Sumatra and its Vicinity, under the Direction of Sir Thomas Stamford Raffles, Lieutenant-Governor of Fort Marlborough; with additional

Notices illustrative of the Natural History of those Countries. *Transactions of the Linnean Society of London*, 13(1), 239-274.

Rajahram, G. S., Barber, B. E., William, T., Menon, J., Anstey, N. M., and Yeo, T. W. (2012). Deaths due to *Plasmodium knowlesi* malaria in Sabah, Malaysia: association with reporting as *Plasmodium malariae* and delayed parenteral artesunate. *Malaria Journal*, 11(1), 1-7.

Reddy, M. P., Sarla, N., and Siddiq, E. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128(1), 9-17.

Ricklefs, R. E., Fallon, S. M., and Bermingham, E. (2004). Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, 53(1), 111-119.

Ruas, P. M., Ruas, C. F., Rampim, L., Carvalho, V. P., Ruas, E. A., and Sera, T. (2003). Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR (Inter-Simple Sequence Repeat) markers. *Genetics and Molecular Biology*, 26(3), 319-327.

Rubio, J., Benito, A., Roche, J., Berzosa, P., Garcia, M., Mico, M., *et al.* (1999). Semi-nested, multiplex polymerase chain reaction for detection of human malaria parasites and evidence of *Plasmodium vivax* infection in Equatorial Guinea. *The American Journal of Tropical Medicine and Hygiene*, 60(2), 183-187.

Sabbatani, S., Fiorino, S., and Manfredi, R. (2010). The emerging of the fifth malaria parasite (*Plasmodium knowlesi*): a public health concern? *Brazilian Journal of Infectious Diseases*, 14(3), 299-309.

Sachs, J., and Malaney, P. (2002). The economic and social burden of malaria. *Nature*, 415(6872), 680-685.

Sandosham, A., Eyles, D., and Yap, L. (1962). *Plasmodium cynomolgi* *bastianellii* from a Malayan monkey and its similarity to aberrant *P. vivax* in man. *Med J Malaya*, 17, 78-79.

Sandoshan, A. (1967). Animal parasites of animals which affect man in Malaysia. *Med. J. Malaya*, 22, 16-25.

- Sato, H., Honma, R., Yoneda, M., Miura, R., Tsukiyama-Kohara, K., Ikeda, F., *et al.* (2008). Measles virus induces cell-type specific changes in gene expression. *Virology*, 375(2), 321-330.
- Schillaci, M. A., Saravia, S., Lee, B. P.-H., and Matheson, C. (2011). Preliminary report on mitochondrial DNA variation in *Macaca fascicularis* from Singapore. *The Raffles Bulletin of Zoology*, 59, 101-108.
- Seethamchai, S., Putapornpip, C., Malaivijitnond, S., Cui, L., and Jongwutiwes, S. (2008). Malaria and *Hepatozoon* species in wild macaques, southern Thailand. *The American Journal of Tropical Medicine and Hygiene*, 78(4), 646-653.
- Singh, B., Bobogare, A., Cox-Singh, J., Snounou, G., Abdullah, M. S., and Rahman, H. A. (1999). A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *The American Journal of Tropical Medicine and Hygiene*, 60(4):687-92
- Singh, B., Sung, L. K., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., *et al.* (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *The Lancet*, 363(9414), 1017-1024.
- Sneath, P. H., and Sokal, R. R. (1973). Numerical taxonomy. The principles and practice of numerical classification. *Numerical Taxonomy. the Principles and Practice of Numerical Classification*, 573.
- Snounou, G., and Singh, B. (2002). Nested PCR analysis of *Plasmodium* parasites *Malaria Methods and Protocols* (pp. 189-203): Springer.
- Snounou, G., Viriyakosol, S., Zhu, X. P., Jarra, W., Pinheiro, L., do Rosario, V. E., *et al.* (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Molecular and Biochemical Parasitology*, 61(2), 315-320.
- Sokal, R. R. (1958). A statistical method for evaluating systematic relationships. *University Kansas Science Bulletin*, 38, 1409-1438.
- Spitzen, J., Spoor, C. W., Grieco, F., ter Braak, C., Beeuwkes, J., van Brugge, S. P., *et al.* (2013). A 3D analysis of flight behavior of *Anopheles gambiae* sensu stricto malaria mosquitoes in response to human odor and heat. *PLoS ONE*, 8(5), e62995.

- Stearns, S. C., and Koella, J. C. (2007). Evolution in health and disease. *Oxford University Press*, 79(4)
- Su, Hayton, K., and Wellems, T. E. (2007). Genetic linkage and association analyses for trait mapping in *Plasmodium falciparum*. *Nature Reviews Genetics*, 8(7), 497-506.
- Su, Jiang, H., Yi, M., Mu, J., and Stephens, R. M. (2009). Large-scale genotyping and genetic mapping in *Plasmodium* parasites. *The Korean Journal of Parasitology*, 47(2), 83-91.
- Su, Kirkman, L. A., Fujioka, H., and Wellems, T. E. (1997). Complex polymorphisms in an ~ 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. *Cell*, 91(5), 593-603.
- Su, and Wellems, T. E. (1996). Toward a high-resolution *Plasmodium falciparum* linkage map: polymorphic markers from hundreds of simple sequence repeats. *Genomics*, 33(3), 430-444.
- Sutherland, C. J., Tanomsing, N., Nolder, D., Oguike, M., Jennison, C., Pukrittayakamee, S., et al. (2010). Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *Journal of Infectious Diseases*, 201(10), 1544-1550.
- Tan. (2008). Identification of vectors of *Plasmodium knowlesi* and other malaria parasites, and studies on their bionomics in Kapit, Sarawak, Malaysia. MSc. Thesis *University Malaysia, UNIMAS, Sarawak*.
- Tan, Vythilingam, I., Matusop, A., Chan, S. T., and Singh, B. (2008). Bionomics of *Anopheles latens* in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*. *Malaria Journal*, 7, 52.
- Tang, T.-H. T., Salas, A., Ali-Tammam, M., Martínez, M. C., Lanza, M., Arroyo, E., et al. (2010). First case of detection of *Plasmodium knowlesi* in Spain by Real Time PCR in a traveller from Southeast Asia. *Malaria Journal*, 9(1), 219.
- Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, 17(16), 6463-6471.

- Thierry, B., Singh, M., and Kaumanns, W. (2004). *Macaque societies: a model for the study of social organization* (Vol. 41): Cambridge University Press.
- Tiedemann, R., Moll, K., Paulus, K., and Schlupp, I. (2005). New microsatellite loci confirm hybrid origin, parthenogenetic inheritance, and mitotic gene conversion in the gynogenetic Amazon molly (*Poecilia formosa*). *Molecular Ecology Notes*, 5(3), 586-589.
- Tong, J., Wang, Z., Yu, X., Wu, Q., and Chu, K. (2002). Cross-species amplification in silver carp and bighead carp with microsatellite primers of common carp. *Molecular Ecology Notes*, 2(3), 245-247.
- Tosi, A. J., Morales, J. C., and Melnick, D. J. (2003). Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. *Evolution*, 57(6), 1419-1435.
- Trampuz, A., Jereb, M., Muzlovic, I., and Prabhu, R. M. (2003). Clinical review: Severe malaria. *Critical Care-London-*, 7(4), 315-323.
- Udwadia, Z. F., Amale, R. A., Ajbani, K. K., and Rodrigues, C. (2012). Totally drug-resistant tuberculosis in India. *Clinical Infectious Diseases*, 54(4), 579-581.
- Valdiani, A., Talei, D., Javanmard, A., Tan, S. G., Kadir, M. A., and Maziah, M. (2014). Morpho-molecular analysis as a prognostic model for repulsive feedback of the medicinal plant "Andrographis paniculata" to allogamy. *Gene*, 542(2), 156-167.
- Vythilingam, I. (2010). Review paper *Plasmodium knowlesi* in humans: a review on the role of its vectors in Malaysia. *Tropical Biomedicine*, 27(1), 1-12.
- Vythilingam, I., Foo, L., Chiang, G., Chan, S., Eng, K., Mahadevan, S., et al. (1995). The impact of permethrin impregnated bednets on the malaria vector *Anopheles maculatus* (Diptera: Culicidae) in aboriginal villages of Pos Betau Pahang, Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 26(2), 354.
- Vythilingam, I., NoorAzian, Y. M., Huat, T. C., Jiram, A. I., Yusri, Y. M., Azahari, A. H., et al. (2008). *Plasmodium knowlesi* in humans, macaques and mosquitoes in peninsular Malaysia. *Parasit Vectors*, 1(1), 26.
- Vythilingam, I., Tan, C., Asmad, M., Chan, S., Lee, K., and Singh, B. (2006). Natural transmission of *Plasmodium knowlesi* to humans by *Anopheles*

latens in Sarawak, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 100(11), 1087-1088.

Walliker, D., Quakyi, I. A., Wellems, T. E., McCutchan, T. F., Szarfman, A., London, W. T., *et al.* (1987). Genetic analysis of the human malaria parasite *Plasmodium falciparum*. *Science*, 236(4809), 1661-1666.

Wang, C. Y., Finstad, C. L., Walfield, A. M., Sia, C., Sokoll, K. K., Chang, T.-Y., *et al.* (2007). Site-specific UBITH amyloid- β vaccine for immunotherapy of Alzheimer's disease. *Vaccine*, 25(16), 3041-3052.

Warrell, D. A., and Gilles, H. M. (2002). *Essential Malariology*: Arnold.

Warren, M., Cheong, W., Fredericks, H., and Coatney, G. R. (1970). Cycles of jungle malaria in West Malaysia. *American Journal of Tropical Medicine and Hygiene*, 19(3), 383-393.

Weber, J. L., and May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, 44(3), 388.

Wellems, T. E., Panton, L. J., Gluzman, I. Y., Do Rosario, V. E., Gwadz, R. W., Walker-Jonah, A., *et al.* (1990). Chloroquine resistance not linked to *mdr*-like genes in a *Plasmodium falciparum* cross. *Nature*, 345, 253 - 255.

Wharton, R., and Eyles, D. E. (1961). *Anopheles hackeri*, a vector of *Plasmodium knowlesi* in Malaya. *Science*, 134(3474), 279-280.

White, N. (2008). *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Diseases*, 46(2), 172-173.

WHO. (2008). *World malaria report 2008*: World Health Organization.

WHO. (2012). *World malaria report 2012*.

WHO. (2013). *Malaria entomology and vector control*: World Health Organization.

Wieczorek, G., Bigaud, M., Menninger, K., Riesen, S., Quesniaux, V., Schuurman, H. J., *et al.* (2006). Acute and chronic vascular rejection in nonhuman primate kidney transplantation. *American Journal of Transplantation*, 6(6), 1285-1296.

- William, T., Rahman, H. A., Jelip, J., Ibrahim, M. Y., Menon, J., Grigg, M. J., *et al.* (2013). Increasing incidence of *Plasmodium knowlesi* malaria following control of *P. falciparum* and *P. vivax* Malaria in Sabah, Malaysia. *PLoS Neglected Tropical Diseases*, 7(1), e2026.
- Wu, K.-s., Jones, R., Danneberger, L., and Scolnik, P. A. (1994). Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Research*, 22(15), 3257-3258.
- XIAO, L. Q., GE, X. J., Gong, X., Hao, G., and ZHENG, S. X. (2004). ISSR variation in the endemic and endangered plant *Cycas guizhouensis* (Cycadaceae). *Annals of Botany*, 94(1), 133-138.
- Yegneswaran, B., Alcid, D., and Mohan, J. (2009). *Plasmodium knowlesi: An Important Yet Overlooked Human Malaria Parasite*. Paper presented at the Mayo Clinic Proceedings.
- Zane, L., Bargelloni, L., and Patarnello, T. (2002). Strategies for microsatellite isolation: a review. *Molecular Ecology*, 11(1), 1-16.
- Zardoya, R., Vollmer, D. M., Craddock, C., Streelman, J. T., Karl, S., and Meyer, A. (1996). Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (*Pisces Perciformes*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263(1376), 1589-1598.
- Zietkiewicz, E., Rafalski, A., and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20(2), 176-183.

LIST OF PUBLICATIONS

Mehdi Kargar, Mohammad Zareain Jahromi , Mahmood Najafian, Parastoo Khajeaian, Reza Nahavandi, Sareh Raeiszadeh Jahromi and Mohammad Firoozinia. (2012). Identification and molecular analysis of mercury resistant bacteria in Kor River, Iran. *African Journal of Biotechnology*, 11(25), 6710-6717.

Mahmood Najafian, Mohammad Zareain Jahromi, Mohammad Javad Nowroznejhad, Parastoo Khajeaian, Mohammad Mehdi Kargar, Mehdi Sadeghi, Amir Arasteh. (2012). Phloridzin reduces blood glucose levels and improves lipids metabolism in streptozotocin-induced diabetic rats. *Molecular Biology Reports*, 39(5), 5299-5306.

Nikzad, Sonia, Soon Guan Tan, Christina Yong Seok Yien, Jillian Ng, Noorjahan Banu Alitheen, Razib Khan, Jeffrine J. Rovie-Ryan, Alireza Valdiani, Parastoo Khajeaian, and Sree Kanthaswamy. (2014). Genetic diversity and population structure of long-tailed macaque (*Macaca fascicularis*) populations in Peninsular Malaysia. *Journal of Medical Primatology*.

ISSRs: Neglected DNA Markers for Molecular Dissection of *Plasmodium* Species in Long-tailed Macaque (*Macaca fascicularis*) Parastoo Khajeaian¹, Soon Guan Tan¹, Alireza Valdiani^{2*}, Christina Yong Seok Yien³, Mohammad Zareian Jahromi⁴, Noorjahan Banu Alitheen¹, Jeffrine Japning Rovie-Rian⁵ *BioMed Research International* (Under Review)



UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

NAME OF STUDENT : _____

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (✓)

CONFIDENTIAL

(Contain confidential information under Official Secret Act 1972).

RESTRICTED

(Contains restricted information as specified by the organization/institution where research was done).

OPEN ACCESS

I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

PATENT

Embargo from _____ until _____
(date) (date)

Approved by:

(Signature of Student)
New IC No/ Passport No.:

(Signature of Chairman of Supervisory Committee)
Name:

Date :

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]