

CONTENTS

	Page
Acknowledgement	d
Abstract in Thai	f
Abstract in English	i
List of Tables	n
List of Figures	q
List of Abbreviations and Symbols	u
Statement of Originality in Thai	v
Statement of Originality in English	w
Chapter 1 Introduction	1
1.1 Statement and Significance of the Problem	1
1.2 Literature Review	3
1.2.1 Distributions and medical important	3
1.2.2 Mating behavior	4
1.3 Purpose of This Study	7
1.4 Usefulness of the Study	7
Chapter 2 Materials and Methods	9
2.1 Materials	9
2.1.1 Molecular study	9
2.1.2 Mosquito rearing	10
2.1.3 Measurement	10
2.2 Chemicals	11
2.2.1 Molecular study	11
2.2.2 Measurement	11

CONTENTS (continued)

	Page
2.2.3 Scanning electron microscopy (SEM)	11
2.3 Methods	12
2.3.1 Field collection of the Hyrcanus Group	12
2.3.2 Morphological species identification	12
2.3.3 Molecular species identification	14
2.3.4 Mosquito rearing procedures	15
2.3.5 Establishment of a stock colony	16
2.3.6 Screening for stenogamous behavior and the establishment of self-mating colony	16
2.3.7 Searching for possible mechanism(s) that control stenogamous mating	17
1) Mating ability of adult mosquitoes in various sized cubic cages and a density resting surface (DRS)	17
2) Measurements and large sensilla coeloconica counts under light and scanning electron microscopy (SEM)	20
3) Frequency of clasper movement in male genitalia during induced copulation, and mating times	24
2.4 Statistical analysis	26
Chapter 3 Results	27
3.1 Molecular species identification	27
3.2 Establishment of a stock colony	32
3.3 Screening of stenogamous behavior and establishment of self-mating colonies	32
3.4 Searching for possible mechanism(s) that control stenogamous mating	34

CONTENTS (continued)

	Page
3.4.1 Mating ability of adult mosquitoes in various sized cubic cages and a density resting surface (DRS)	34
1) Mating ability of adult mosquitoes in 10, 20 and 30 cubic cm cages at DRS of 3.6	34
2) Mating ability of adult mosquitoes in 10, 20, 30 and 40 cubic cm cages at DRS of 7.2	38
3.4.2 Measurements and large sensilla coeloconica counts under light and scanning electron microscopy (SEM)	44
1) Measurements of wings in adult females and males	44
2) Measurements of maxillary palpomeres of females	47
3) Number and form of large sensilla coeloconica on antennae of females	49
4) Measurements of male genitalia	55
5) Clasper movement and duration of mating	61
Chapter 4 Discussion	62
4.1 Molecular species identification	62
4.2 Screening of stenogamous behavior and establishment of self-mating colony	63
4.3 Searching for possible mechanism(s) that control stenogamous behavior	64
Chapter 5 Conclusion	69
References	70
List of Publications	81
Appendix	82
Curriculum Vitae	100

LIST OF TABLES

		Page
Table 3.1	Mean intra- and inter- specific genetic distances (K2P) for eight species of the Hyrcanus Group based on COI barcode sequences	29
Table 3.2	The insemination rates of selective stenogamous-colonies of <i>An. peditaeniatus</i> , <i>An. paraliae</i> , <i>An. nigerrimus</i> , <i>An. pursati</i> and <i>An. sinensis</i>	33
Table 3.3	Insemination rates of the eight species of the Hyrcanus Group using various cage sizes (10, 20 and 30 cubic cm cages) at DRS of 3.6	35
Table 3.4	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (10 cubic cm cage, DRS = 3.6)	36
Table 3.5	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (20 cubic cm cage, DRS = 3.6)	37
Table 3.6	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (30 cubic cm cage, DRS = 3.6)	38

LIST OF TABLES (continued)

		Page
Table 3.7	Insemination rates of the eight species of the Hyrcanus Group using various cage sizes (10, 20, 30 and 40 cubic cm cages) at DRS of 7.2	39
Table 3.8	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (10 cubic cm cage, DRS = 7.2)	40
Table 3.9	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (20 cubic cm cage, DRS = 7.2)	41
Table 3.10	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (30 cubic cm cage, DRS = 7.2)	42
Table 3.11	Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (40 cubic cm cage, DRS = 7.2)	43
Table 3.12	Comparisons of mean wing ratios of females and males of the eight species of the Hyrcanus Group (30 females or males/species, $n = 60$)	45

LIST OF TABLES (continued)

		Page
Table 3.13	Comparisons of the mean palpomeres ratios of females of the eight species of the Hyrcanus Group (30 females/species, $n = 60$)	48
Table 3.14	Mean distributions of sensilla coeloconica on the 13 antennal flagellomeres of females of the six species of the Hyrcanus Group (30 females/species, $n = 60$)	54
Table 3.15	Comparisons of measurements (length and width in microns) of male genital structures in the eight species of the Hyrcanus Group (30 males/species)	56
Table 3.16	Comparisons of the results of statistical analyses of male genital measurements for the eight species of the Hyrcanus Group	58
Table 3.17	Comparative measurements of mating time (duration in seconds) and frequency of clasper movement per copulation in the eight species of the Hyrcanus Group ($n = 30$)	61

LIST OF FIGURES

	Page	
Figure 1.1	Sequences in the coupling of <i>Anopheles gambiae</i> s.s., drawn from photographs of males coupling with females suspended from thorax (Charlwood and Jones 1979)	5
Figure 2.1	Map of Thailand showing six provinces where samples of eight species belonging to the Hyrcanus Group were collected	13
Figure 2.2	Four cages (left to right: 10, 20, 30 and 40 cubic cm cages) used for self-mating mosquitoes	18
Figure 2.3	Grading of sperm within spermatheca of inseminated females of the eight species. A, 0; B, 1+; C, 2+; D, 3+ and E, 4+ (100x magnification)	19
Figure 2.4	The measurement of wing length and width of the eight female species. Scale bars: 0.2 mm	20
Figure 2.5	Schematic of the head (female anopheline). A, antenna; C, clypeus; L, labium (proboscis); Lb, labella; m, tip of mouthparts lying in labial sheath; P, palp (segments 1- 5) (modified from Reid 1968)	21

LIST OF FIGURES (continued)

	Page	
Figure 2.6	Schematic measurements of the male genitalia (ventral view) at various sites. A, length of the aedeagus; B, width of the aedeagus; C, length between the base of the aedeagus and origin of the gonocoxite; D, width of the gonocoxite at the origin of the parabasal setae; E, Length of the gonocoxite; F, length of the gonostylus (modified from Harrison and Scanlon 1975)	22
Figure 2.7	Representative female antennae of the eight species of the Hyrcanus Group. A, flagellomeres are typically numbered 1-13 from one pair of female antenna; B, large sensilla coeloconica (pitted peg) (arrow) on each flagellomere. Scale bars: 0.01 mm for A; 0.02 mm for B	23
Figure 2.8	Summarized experimental design	25
Figure 3.1	Bootstrapped neighbor-joining tree (NJ) of the COI barcode sequences of nine species of the Hyrcanus Group. <i>Anopheles gambiae</i> and <i>Anopheles braziliensis</i> were used as outgroup taxa. Bootstrap values higher than 70% are shown above the node. Bars represent 0.01 substitutions per site	30

LIST OF FIGURES (continued)

	Page	
Figure 3.2	Bayesian phylogenetic tree based on COI barcode sequences of nine species of the Hyrcanus Group. <i>Anopheles gambiae</i> and <i>An. braziliensis</i> were used as outgroup taxa. The posterior probabilities higher than 70% is shown above the nodes. Bars represent 0.05 substitutions per site	31
Figure 3.3	The cluster of large sensilla coeloconica located in a sunken depression (sacculus) on the 5 th antennal flagellomere of <i>An. argyropus</i> (A) and <i>An. peditaeniatus</i> (B), photographed under a light microscope. Scale bar: 0.02. mm	50
Figure 3.4	Scanning electron micrographs of the 5 th antennal flagellomere of <i>An. peditaeniatus</i> : (A) sacculus (circle) containing large sensilla coeloconica (1,000x); (B) higher magnification of the sacculus with its sensilla coeloconica (5,000x). The different types of sensilla shown in the micrographs occur on most flagellomeres: lch, large chaetica; sch, small chaetica; st, sharp trichoid; lco, large coeloconica and gp, grooved peg	51

LIST OF FIGURES (continued)

	Page	
Figure 3.5	The distribution of large sensilla coeloconica on the 5 th antennal flagellomere of the six species of the Hyrcanus Group, photographed under a light microscope: A, <i>An. crawfordi</i> ; B, <i>An. nitidus</i> ; C, <i>An. nigerrimus</i> ; D, <i>An. paraliae</i> ; E, <i>An. pursati</i> and F, <i>An. sinensis</i> . Scale bar: 0.02 mm	52
Figure 3.6	Scanning electron micrographs of the 5 th antennal segment of <i>An. paraliae</i> . A, showing large sensilla coeloconica (x 1,000) and B, a higher magnification of large sensilla coeloconica (x 4,000). The different types of sensilla shown in the micrographs occur on most flagellomeres: lch, large chaetica; sch, small chaetica; st, sharp trichoid; bt, blunt trichoid; lco, large coeloconica and gp, grooved peg	53

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
µl	Microliter
µM	Micromolar
bp	Base pair
cm	Centimeter
COI	Cytochrome <i>c</i> oxidase subunit I
COII	Cytochrome <i>c</i> oxidase subunit II
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
e.g.	Exempli gratia
et al	And others
etc.	Etcetera
i.e.	Id est
ITS2	Second internal transcribed spacer
ml	Milliliter
mM	Millimolar
mtDNA	mitochondrial DNA
PCR	Polymerase chain reaction
pH	Potential of hydrogen
rDNA	ribosomal DNA
U	Unit

ข้อความแห่งการริเริ่ม

1. วิทยานิพนธ์นี้เป็นการศึกษาแรกที่นำขึ้นในโடค่อนเดรียในตำแหน่ง cytochrome *c* oxidase subunit I (COI) มาใช้เป็นคิเอ็นเอobar์ โคดในการจำแนกชนิดยุงกั้นปล่องที่เป็นสมาชิกในกลุ่ม ไคร์คานัส จำนวน 8 ชนิด ในประเทศไทย ได้แก่ *Anopheles argyropus*, *Anopheles crawfordi*, *Anopheles nigerrimus*, *Anopheles nitidus*, *Anopheles paraliae*, *Anopheles peditaeniatus*, *Anopheles pursati* และ *Anopheles sinensis*
2. วิทยานิพนธ์นี้เป็นการศึกษาแรกที่ทำการตรวจหาพฤติกรรมผสมพันธุ์ในที่แคนของยุงกั้นปล่อง กลุ่ม ไคร์คานัสทั้ง 8 ชนิดในประเทศไทย และแสดงให้เห็นถึงการประสบความสำเร็จในการตั้ง โคโลนียุงกั้นปล่อง *An. peditaeniatus* ที่มีพฤติกรรมผสมพันธุ์ในที่แคน
3. วิทยานิพนธ์นี้เป็นการศึกษาแรกที่แสดงให้เห็นถึงกลไกที่ควบคุมพฤติกรรมผสมพันธุ์ในที่แคน ของยุงกั้นปล่อง *An. peditaeniatus* ในประเทศไทย

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright[©] by Chiang Mai University
All rights reserved

STATEMENT OF ORIGINALITY

1. This thesis is on the first study to utilize mitochondrial cytochrome *c* oxidase subunit I (COI) gene-based DNA barcoding, for species identification of the eight species, i.e., *Anopheles argyropus*, *Anopheles crawfordi*, *Anopheles nigerrimus*, *Anopheles nitidus*, *Anopheles paraliae*, *Anopheles peditaeniatus*, *Anopheles pursati*, and *Anopheles sinensis*, belonging to the Hyrcanus Group in Thailand.
2. This thesis is on the first study to screen the stenogamous behavior of the eight species belonging to the Hyrcanus Group in Thailand and demonstrate the successful establishment of a stenogamous colony of *An. peditaeniatus*.
3. This thesis is on the first study to demonstrate the mechanisms that control stenogamous behavior of *An. peditaeniatus* in Thailand.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright[©] by Chiang Mai University
All rights reserved