

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT (IN ENGLISH)	v
ABSTRACT (IN THAI)	vii
TABLE OF CONTENTS	ix
LIST OF TABLES	xi
LIST OF FIGURES	xii
ABBREVIATIONS AND SYMBOLS	xvii
CHAPTER I INTRODUCTION	1
1. STATEMENT AND SIGNIFICANCE OF THE PROBLEM	1
2. LITERATURE REVIEWS	2
3. OBJECTIVE	18
4. USEFULNESS OF THE STUDY	18
CHAPTER II MATERIALS AND METHODS	19
1. MATERIALS	19
1.1 For preparation of blood containing <i>B. malayi</i> microfilariae	19
1.2 For light and scanning electron microscopic studies	19

TABLE OF CONTENTS (continued)

	Page
2. METHODS	19
2.1 Mosquito rearing	19
2.2 Preparation of blood containing <i>B. malayi</i> microfilariae and infection of mosquitoes	22
2.3 Exsheathment studies and preparation of samples for light microscopy (LM)	23
2.4 Preparation of samples for scanning electron microscopy (SEM)	23
CHAPTER III RESULTS	25
1. Analysis of exsheathment and midgut invasion of <i>B. malayi</i> microfilariae in <i>Ae. aegypti</i> using LM	25
2. Analysis of exsheathment and midgut invasion of <i>B. malayi</i> microfilariae in <i>Ae. aegypti</i> using SEM	29
CHAPTER IV DISCUSSION	34
CHAPTER V CONCLUSION	39
REFERENCES	40
APPENDIX	49
CURRICULUM VITAE	50

LIST OF TABLES

Table		Page
3.1	Average number and percentage of microfilariae (mff) of NSP <i>B. malayi</i> in the midgut and hemolymph in <i>Ae. aegypti</i> at different times PIBM based on 30 infected mosquitoes per time point from triplicate experiments (n=10/mosquito/time point/experiment; 450 total)	28

LIST OF FIGURES**Figure**

- 1.1 World map shows the global distribution and status of mass drug administration for lymphatic filariasis, 2011 (Modified from http://gamapserver.who.int/mapLibrary/Files/Maps/LF_2011.png). 4
- 1.2 Province and villages affected with lymphatic filariasis in Thailand, 2014 (Modified from <http://www.thaiibd.org/n/contents/view/324451>). 5
- 1.3 Life cycle of filarial parasite. (a) The infective third-stage filarial larva (L3) enters the host when a mosquito takes a blood meal. (b) The L3 migrates to lymphatic vessels and develops into the L4 stage, to the young adult stage and finally to the mature adult worm, male or female that commonly reside in the lymphatic system. (c) After mating, the gravid female produces sheathed microfilariae into lymph and enter the bloodstream reaching the peripheral blood. (d) After a mosquito acquires the infection by ingestion of an infected blood meal, microfilaria penetrates midgut wall and traverses the hemocoel 8

LIST OF FIGURES (continued)

Figure	Page
<p>to invade the thoracic muscle cells and then develops to the L3 which migrates to the head and proboscis and enters a new host by penetrating the labellum of the proboscis (Modified from http://intranet.tdmu.edu.ua/data/kafedra/internal/med_biologia/classes_stud/en/med/lik/ptn/medical%20biology/1%20course/Theme%2010.htm).</p>	8
<p>1.4 Morphology of different stages of <i>B. malayi</i>. (a) Microfilaria is ingested during blood feeding. (b) Parasite differentiates into non-feeding, L1 within mosquito indirect flight muscle cells. (c) After the first molt, L2 remains intracellular parasites which ingest cellular material into its newly developed digestive tract. (d) L3 leaves the muscle cells and migrates to the mosquito's head and proboscis where they will exit through the mosquito cuticle during blood feeding (Modified from Sara et al. 2009). (e) <i>B. malayi</i> adults. The adult male worm is considerably smaller than the female (Modified from http://www.metapathogen.com/lymphatic-filariasis/).</p>	9

LIST OF FIGURES (continued)

Figure		Page
1.5	Fractured midguts and peritrophic matrix (PM) of <i>O. togoi</i> female after taking a <i>B. malayi</i> -infected blood meal (PIBM). At 5h PIBM, SEM micrograph shows an external face of the PM (<i>PMe</i>) that encloses the blood meal (<i>Bl</i>). The PM is completely formed and can be separated from the epithelium (<i>Ep</i>) (Modified from Jariyapan et al. 2013).	16
1.6	SEM micrographs of fractured abdominal midguts showing the invasion process of microfilariae (<i>mf</i>) from the midgut lumen into hemocoel. At 3 h PIBM, SEM showing a sheathed microfilaria (<i>mf</i> ; arrow) penetrating across the internal face of the PM (<i>PM</i>) and epithelium (<i>Ep</i>) into hemocoel in the final stage of invasion (Modified from Jariyapan et al. 2013).	17
2.1	Equipment for mosquito rearing. (a) Plastic cup of natural water with wet filter paper lining the inside for gravid female mosquitoes to lay eggs. (b) Eggs placed in a white plastic tray containing 1,500 ml of natural water and exposed to a 40 watt light. (c) White plastic rearing	21

LIST OF FIGURES (continued)

Figure	Page
tray containing natural water, transfer pipette and dog food. (d) Plastic container for holding pupae (e) Adult rearing cage and bottle with cotton wick containing 10% sucrose solution. (f) Plastic container and sucker. hemolymph.	21
3.1 Representative images of <i>B. malayi</i> microfilariae with intact morphology in <i>Ae. aegypti</i> . (a) A sheathed microfilaria in the midgut. Arrows indicate the microfilarial sheath. (b) An exsheathed microfilaria in the midgut. (c) An exsheathed microfilaria in the	27
3.2 SEM micrographs of abdominal midguts showing the exsheathment of microfilariae. (a) A representative image of a sheathed microfilariae (arrow) surrounded by small particles in the midgut lumen observed in a midgut dissected at 1 h PIBM. (b-d) Representative examples of maceration of the sheaths (arrows) of microfilariae in midguts dissected at 3 h PIBM. (e-f) Representative examples of exsheathed microfilariae (arrows) inside the blood meal (<i>BM</i>) in the midgut dissected at 3 h PIBM.	30
3.3 SEM micrographs of fractured abdominal midguts showing the invasion process of microfilariae. Specimens are from the midgut	31

LIST OF FIGURES (continued)

Figure	Page
<p>lumen and hemocoel at 4 h PIBM. (a,b) Exsheathed microfilariae (arrows) close to the PM. (c) Exsheathed microfilariae (arrows) crossing the PM. (d) Fractured region showing exsheathed microfilariae (arrow) during invasion into the PM. (e) An exsheathed microfilaria (arrow) penetrating across the PM and epithelium into the hemocoel and lying on the external surface (<i>Ex</i>) of the midgut.</p>	31
<p>3.4 SEM micrographs showing sheathed microfilariae in the midgut lumen. (a-f) Representative examples from midguts dissected at 1 h, 3 h, 12 h, 24 h, and 36 h PIBM, respectively.</p>	32
<p>3.5 An example of a melanized microfilaria recovered from the hemocoel. LM showing melanization occurred on a part of the microfilaria body (arrow).</p>	33

ABBREVIATIONS AND SYMBOLS

LM	light microscope
SEM	scanning electron microscope
NSP	nocturnally subperiodic
PIBM	post feeding on a <i>Brugia malayi</i> -infected blood meal
HBSS	Hank's balanced salt solution
PBS	phosphate buffer saline solution
PM	peritrophic matrix
cm	centimeter
ml	milliliter
μl	microliter
h	hour
min	minute
<i>et al</i>	and others

<i>i.e.</i>	id est
%	percentage
°C	degree Celsius