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Figure 1.1 Countries with ongoing transmission of malaria, 2013

Figure 1.2 Life cycle of the *Plasmodium* parasite in its mosquito vector. Female (1) and male (2) gametocytes differentiate into gametes (3 and 4). After completion of meiosis, the male gametocyte generates eight gametes (4) in a process known as 'exflagellation'. A male gamete fertilizes a female gamete (5) to generate a zygote (6), which in turn differentiates into a motile ookinete (7). About 24 hr later, the mature ookinete first traverses the peritrophic matrix (orange line) and then the midgut epithelium (8), after which it differentiates into an oocyst (9). During the next 10 days the oocyst grows (10 and 11) and when mature, it releases sporozoites into the open haemolymph circulation (12). The circulating sporozoites recognize and invade the salivary glands (13) where they are stored until released at the time when the mosquito bites the next individual

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Figure 1.3 Progression of sporozoite invasion of the salivary gland (1) The sporozoite attaches to the basal lamina; (2) sporozoite passage to the space between the basal lamina and basal epithelial cell plasma membrane, a process associated with loss of the sporozoite's thick coat; (3) penetration of the basal plasma membrane; the sporozoite resides within a vesicle; (4) release of the sporozoite from the surrounding membrane by an unknown mechanism; (5) invasion of the apical membrane and entry into the secretory cavity; (6) sporozoites are released from the surrounding membrane by an unknown mechanism; (7) sporozoites assemble into bundles within the secretory cavity; and (8) a small number of sporozoites enter the secretory duct by an unknown mechanism. BL: basal lamina; DW: duct wall; N: nucleus; n: nucleolus; PM: plasma membrane; SC: secretory cavity

Figure 2.1 Experimental design for the first and the second blood feeding of *An. campestris*-like

- Figure 3.1 Females and males salivary gland protein profiles of *An. campestris*-like. The salivary gland proteins were separated on 15 % SDS-polyacrylamide gels and stained with CBB. M: sixty male salivary glands; PL: fifty female proximal lateral lobes; ML: two median lobes; DL: two distal-lateral lobes; F: two whole female salivary glands. Molecular mass markers are indicated on the left in kilodalton. Arrows indicate major salivary gland proteins of female mosquitoes
- Figure 3.2 Two-dimensional gel analysis of female salivary gland proteins of *An. campestris*-like. Proteins were separated on Immobiline DryStrips 7 cm, pI 3–10. Separation in the second dimension was performed using 15 % SDS-PAGE. Molecular mass markers are indicated on the left in kilodalton. Isoelectric points (pI) are indicated at the top. Numbers indicate major salivary gland proteins. a Representative of 2-DE gels stained with CBB; b representative of 2-DE gels stained with Pro-Q Emerald 300 glycoprotein stain

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Figure 3.3 Two-dimensional gel analysis of proteins expressed in male and different regions of female *An. campestris*-like salivary glands. Proteins were separated on Immobiline DryStrips 7 cm, pI 3–10. Separation in the second dimension was performed using 15 % SDS-PAGE. The gels were stained with CBB. Molecular mass markers are indicated on the left in kilodalton. Isoelectric points (pI) are indicated at the top. a Representative of 2-DE gels of proteins extracted from male glands; b proximal-lateral lobes; c medial lobes; d distal-lateral lobes. Circle, a major spot found in distallateral lobe, square, a major spot found in medial lobe, triangle, a major spot found in male, proximal-lateral and distal-lateral lobes, octagon, a major spot found in medial and distal-lateral lobes, diamond, a major spot found in male and all female lobes

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- Figure 3.4 Comparison of 2-DE protein profiles of female salivary gland proteins of An. campestris-like. Proteins were separated on Immobiline Dry Strips 7 cm, pI 3-10. Separation in the second dimension was performed using 15% SDS-PAGE. The gels were stained with Coomassie blue. Molecular mass markers are indicated on the left in kDa. Isoelectric points (pI) are indicated at the top. Numbers indicate major salivary gland proteins. a representative of 2-DE gels of salivary gland proteins of unfed females from the first blood meal group; b salivary gland proteins of blood fed females the first blood meal group; c salivary gland proteins of unfed females from the second blood meal group; d salivary gland proteins of blood fed females the second blood meal group; e salivary gland proteins of unfed females from sugar fed control group. Circle indicates an internal control protein
- Figure 3.5 An. campestris-like female salivary gland cDNA Library clone
- Figure 3.6 NJ Phylogenetic analyses of the salivary proteins in the D7 family of *Anopheles* mosquitoes. The numbers on the branches represent the percentage of bootstrap support. The bar on the bottom represents 10% nucleotide sequence divergence

LIST OF ABBREVIATIONS AND SYMBOLS

WHO	World Health Organization
М	Molar
mM	Millimolar
mg	Milligram
ml	Milliliter
cm 6	Centimeter
min	Minute
sp.	Species
μl	Microliter
μm	Micrometer
μg	Micro Gram
kDa	Kilo Dalton
snadal	Spot Number
cDNA	Complimentary DNA
pI	Isoelectric points
hr	hour
°C	Degree Celsius
%	Percent
α	Alpha



ข้อความแห่งการริเริ่ม

- 1. วิทยานิพนธ์นี้เป็นการวิเคราะห์โปรตีนของต่อมน้ำลายยุง Anopheles campestris-like เพศผู้ และในแต่ละส่วนของต่อมน้ำลายยุงเพศเมียด้วยวิธีการทางโปรตีโอมมิก
- วิทยานิพนธ์นี้เป็นการศึกษาแรกที่จำแนกโปรตีนในต่อมน้ำลายที่เกี่ยวข้องกับการกินเลือดครั้งที่ สองในวันที่สอดคล้องกับช่วงเวลาของการถ่ายทอดเชื้อระยะสปอโรซอยต์ไปสู่โฮสต์ใหม่ที่เป็น สัตว์เลี้ยงลูกด้วยนม
- วิทยานิพนธ์นี้ได้จำแนกโปรตีนที่หลั่งออกมาอย่างมากจากห้องสมุด cDNA ของต่อมน้ำลายยุง
 An. campestris-like เพศเมียเป็นครั้งแรก



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STATEMENT OF ORIGINALITY

- 1. This thesis analyzes proteins of male *An. campestris*-like and different morphological regions of the female salivary glands by using proteomic approach.
- 2. This thesis is the first to identify the salivary gland proteins that are involved in the second blood feed on a day corresponding to the transmission period of sporozoites to a new mammalian host.
- 3. This thesis identifies cDNA Library clones encoding abundant secreted proteins in *An. campestris*-like female salivary glands for the first time.



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