CHAPTER I

Introduction

1.1 Statement and Significance of the Problem

To date, malaria is a major health problem and the number of human cases is increasing worldwide. Malaria parasites require successful completion of the sporogonic cycle in the midgut and salivary glands of mosquitoes. When the mosquito takes up a blood meal with the parasites that they must penetrate the peritrophic matrix through the epithelium. The parasites evade the immune response and keep on their travel towards the salivary gland where they are transmitted to another host. Generally, a life cycle of *Plasmodium* in the mosquito vectors is approximately 10-21 days depending on parasite species and temperature. Understanding of interactions among the midgut, salivary glands, symbionts, and parasites in mosquito vectors, such as changes of physiological characteristics and microorganisms, may help to explain how parasite development and subsequent transmission occur in the vectors.

In Thailand, *Anopheles dissidens*, (formerly *Anopheles barbirostris* species A1), a member of *Anopheles barbirostris* complex in Thailand (Taai and Harbach 2015), has been reported as a low potential vector of *Plasmodium vivax* (Thongsahuan et al. 2011). Despite its importance as a malaria vector, little information is known about its midgut and salivary glands. Previously, it has only a preliminary analysis of female salivary gland proteins of *An. barbirostris* complex by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Nano-liquid chromatography-mass spectrometry (nanoLC-MS) analysis has revealed only a major protein band matched with a protein involved in blood feeding, gSG6, of *Anopheles gambiae* (Jariyapan et al. 2010). No other study has been performed in this mosquito species. It should be intensively fulfilled systematic investigation on changes during aging and after blood feeding, especially the midgut and salivary glands, where are the essential destinations for *Plasmodium*

development and transmission to a new host. Therefore, explorations of morphological changes under electron microscope, and protein changes using SDS-PAGE and 2-DE should be performed in both tissues as well as bacterial diversity in the mosquito midgut.

1.2 Literature Review

1.2.1 Malaria situation

Malaria is ranked the most important parasitic infection worldwide, with 2.1 billion human cases reported (WHO 2014), and despite years of continual effort, it is still a major cause of death in third-world countries. Malaria parasites require a mosquito vector for transmission. Of the several hundred Anopheles mosquito species worldwide, about 20 are major vectors of human malaria. These are found in tropical areas throughout sub-Saharan Africa, and to a lesser extent South Africa, Southeast Asia, the Pacific Islands, India and Central and South America (Na-Bangchang and Congpuong 2007).

A major requirement for transmission of the parasite is an infected blood meal, which initiates a parasite transmission cycle. Development of the malaria parasite in mosquitoes involves significant interaction between pathogens and the host tissue. Mosquito midgut is the first site of contact for potential pathogens, and also plays a central role in the sporogonic development of malaria parasites; ookinetes must negotiate the midgut environment successfully by avoiding digestive enzymes and developing a peritrophic matrix to penetrate and lodge at the basal surface of the midgut epithelium. In addition, mosquito salivary glands play an important role in parasitic transmission to the vertebrate hosts because malaria sporozoites must navigate a way to reach and invade their final destination, which is the salivary glands in the mosquito host (James 2003). Blood feeding is an organized biological mechanism, which involves the use of anticoagulants that cause severe immune reaction by the host, and minimizes the parasite load for its survival. This is not only an obligatory step, but it also seems to facilitate complete maturation of infection competent sporozoites (Dhar and Kumar 2003).

1.2.2 Malaria life cycle in mosquito stage

Human malaria is transmitted exactly by female Anopheline mosquito. Figure 1.1 shows *Plasmodium* development in a mosquito vector (Molina-Cruz et al. 2013). After the mosquitoes ingest blood with male and female gametocytes. Suddenly, the infected blood remains in the midgut lumen for digestion, the gametocytes are activated and transformed into gametes as male (microgametes) and female gametes (macrogametes). The female gamete is fertilized by male gametes and diploid zygotes are formed that differentiate into motile ookinetes. The ookinetes cross through the midgut epithelium and transform into oocysts on the basal side of the midgut. During this time, each oocyst grows and divides to producing thousands of sporozoites (haploid forms). Approximately 10-16 days, sporozoites in mature oocysts are released into the hemolymph which they travel to and invade the salivary glands. The cycle of human infection restarts when the mosquito feeds a blood meal that inject the sporozoites from the salivary glands into the human bloodstream (Molina-Cruz et al. 2013).

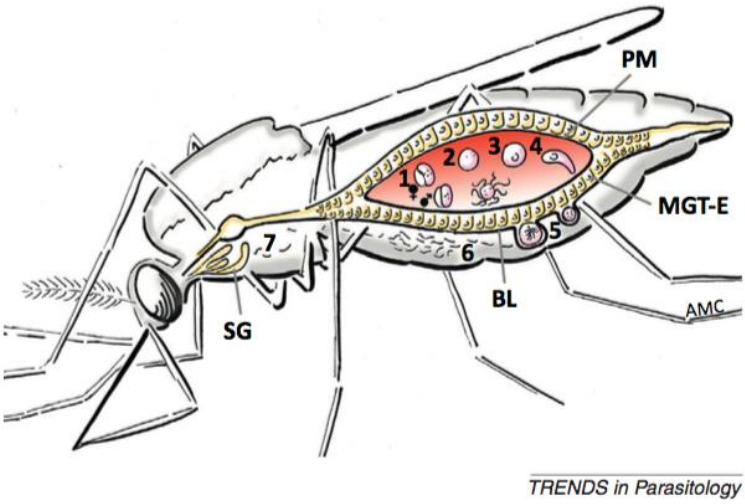

Figure 1.1 *Plasmodium* development in a mosquito vector. Successful transmission of *Plasmodium* spp. by a mosquito involves a complex developmental cycle within the vector. The mosquito must: (1) ingest a blood meal containing male and female *Plasmodium* gametocytes; (2) within minutes the gametocytes develop into gametes; (3) female macrogametes are fertilized by the male microgametes and a diploid zygote is formed; (4) the zygote develops into a motile ookinete; (4) the ookinete crosses the peritrophic matrix (PM) to invade the midgut epithelium (MGT-E) by 16-26 h post-feeding; (5) successful ookinetes traverse the midgut epithelial cells and form oocysts, lying between the basal membrane of the epithelium and the basal lamina (BL); (6) the oocyst takes 7-21 days to develop thousands of sporozoites that are released into the mosquito hemolymph; (7) a fraction of the sporozoites invade the salivary glands (SGs) and remain there to be injected into another vertebrate host when the mosquito takes another blood meal. Each step in the life cycle of *Plasmodium* in the mosquito can potentially represent a barrier for transmission (Molina-Cruz et al. 2013).

1.2.3 Mosquito midgut as the initial site of infection

1) Midgut morphology

Blood-feeding insects can be divided into two groups depending on the design of the alimentary canal for storage of the blood meal (Lehane 2005). In one group, typified by Hemiptera, the alimentary canal is a simple tube with no diverticulae and the blood is stored in the midgut. In the second group, typified by Diptera, the gut has between one and three diverticulae which may be used, in addition to the midgut, for the storage of the blood meal.

The mosquito midgut plays a crucial role because it is the primary tissue involved in processing the blood meal (Romoser 1996). It is responsible not only for digestion and uptake of nutrient, but is also the first site of contact for potential pathogens. Transmission is initiated when the mosquito ingests a blood meal from a parasitemic individual. *Plasmodium* parasites follow a complex developmental program that includes transformation into multiple morphological forms and the crossing of the midgut and salivary gland epithelia (reviewed by Ghosh et al. 2000). Ingestion of a blood meal also triggers a number of dramatic morphological and biochemical changes in the midgut epithelium.

The morphological observations of the mosquito midgut contribute to the understanding of mosquito physiology. The number of morphological studies of the midgut has been described (Hecker 1977; Clements 1992; Siden-Kiamos and Louis 2004; Abraham and Jacobs-Lorena 2004; Okuda et al. 2002, 2005; Cázares-Raga et al. 2014). In general, a mosquito midgut has been divided into 3 major regions constitute the insect's alimentary tube: foregut, midgut, and hindgut. The foregut and hindgut are of ectodermic origin and their cells secrete a chitinous layer continuous with the integumentary cuticle. A narrow anterior and dilated posterior region constitute the midgut, which is of endodermal origin. Its epithelium is composed of a monolayer of adjacent digestive cells that rest on a basal lamina. Regenerative cells scattered between digestive ones distributed at the basal side.

Under electron microscopy, the midgut epithelium of mosquitoes consists of monolayer of columnar polarized cells, which lie on a non-cellular basal lamina. In the apical cytoplasm, many mitochondria, cisterns of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), nucleus (N), free ribosome and vesicle with electron-lucent content (EDV) have been observed. Apical membranes formed microvilli protruded into the midgut lumen. Also, the vesicle with electron-lucent content move toward the apical membrane, where released the content into the midgut.

After blood feeding, engorgement causes great distension of the midgut and secretion of substances synthesizing before blood feeding as peritrophic matrix (PM) precursor granules and digestive enzymes are immediately released (Billingsley 1990), as shown in Figure 1.2. The PM of mosquito have two types, which they have distinct properties. The PM formation becomes compacted as it appears two layers between epithelium cells and blood meal, which is a barrier for parasites and bacteria attempting to invade the midgut epithelium (Okuda et al. 2005). To pass through the gut epithelium, the parasite has to cross the PM by its own enzyme.

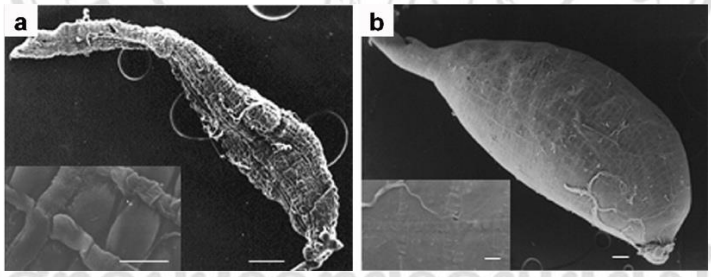

Figure 1.2 SEM images of midgut sugar and blood fed mosquitoes. The images showed the dramatic distension of the midgut mosquito caused by the ingestion of a blood meal. a) midgut of sugar fed mosquito, bar = 500 µm. b) Midgut of blood fed mosquito, bar = 10 µm (Cázares-Raga et al. 2014).

2) Midgut proteins

The number of mosquito midgut proteome has been described in *Anopheles stephensi, An. gambiae, Anopheles albimanus, Aedes aegypti, Aedes albopictus* (Prevot et al. 1998, 2003; Cázares-Raga et al. 2014; Butler et al. 2009; Saboia-Vahia et al. 2012). Characterization of *An. gambiae* midgut proteins by two-dimensional gel electrophoresis (2-DE) has revealed very few differences between male and female mosquitoes (Prévot et al. 2003). In sugar fed of female *Ae. albopictus* mosquito, the midgut proteins could be classified in 5 groups of functional associations. The protein groups have involved in, such as glycolysis, carbohydrate and amino acid metabolism, functional association network, lipid metabolism pathway, and the detoxification of free radicals (Saboia-Vahia et al. 2012).

Moreover, a few studies have afforded to identify parasite/virus receptors in the mosquito midgut as a critical step in understanding vector competence and designing possible targets for preventing parisite/virus entry to midgut cells (Serrano-Pinto et al. 2010; Tchankouo-Nguetcheu et al. 2010). Serrano-Pinto et al. (2010) have analyzed the midgut of *An. albimanus* infected with *Plasmodium berghei* (resistant mosquito), found 21 spot proteins differentially expressed in the blood of mosquitoes during the immune challenge. The differentially expressed proteins have been associated with diverse functions, such as proteins with digestion, immunity, nucleotide binding, transport, protein metabolism, gustatory response, transcriptional regulation, regulator mitosis cell division, translation process, enzymatic activity, and others and unknown proteins. Moreover, Tchankouo-Nguetcheu et al. (2010) have aimed to verify how the same vector responds to different arboviruses at the midgut level.

1.2.4 Mosquito salivary glands as the original site of transmission

1) Salivary gland morphology

Salivary glands of mosquito play an important role in parasitic transmission. A number of morphological studies has described the salivary glands of *Ae. aegypti, An. stephensi, Culex pipiens, An. darlingi,* and *Culex quinquefasciatus* (Orr et al. 1961; Wright 1969; Janzen and Wright 1971; Barrow et al. 1975; Moreira-Ferro et al.

1999; da Cunha Sais et al. 2003). However, histological sections of adult female salivary glands related to the age of mosquitoes have only been studied in *Ae. aegypti* and *Aedes togoi* (Beckett, 1990).

The salivary glands of adult mosquitoes are sexually dimorphic and their different morphology and function clearly enable females to engage successfully in hematophagy (Stark and James 1996). The salivary glands of male mosquitoes are smaller than the female salivary glands and male salivary glands have only one lobe (Figure 1.3). The salivary glands of adult female *Anopheles* mosquitoes are similar, consisting of a distinctive three lobes connected to a main salivary duct, a single medial and two lateral lobes (Figure 1.3). Each lobe comprises a secretory epithelium surrounding a salivary duct into which saliva is released (Figure 1.3).

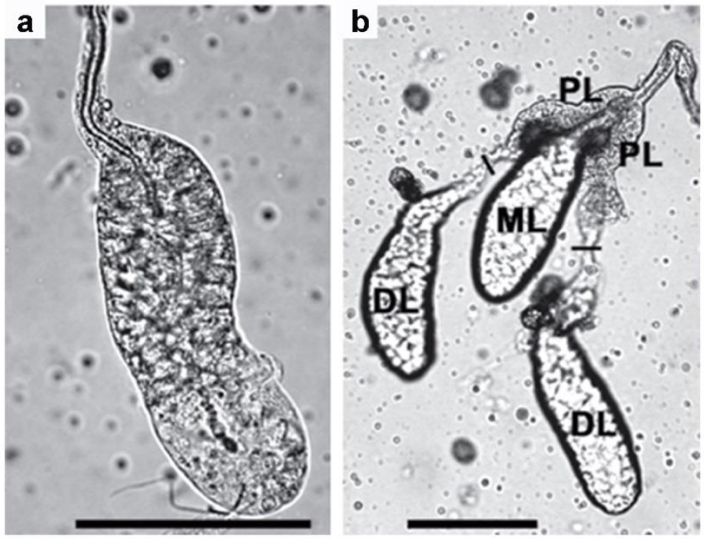

Figure 1.3 Representative adult salivary glands of the mosquito, *Anopheles dirus* B. a) A male salivary gland. b) a female salivary gland. PL: proximal region of the lateral lobe; DL: distal region of the lateral lobe; ML: median lobe. Bar represents 500 µm (Jariyapan et al. 2007).

2) Salivary gland proteins

Mosquitoes saliva plays important roles in lubricating mouthparts, solubilizing and initially digesting blood (Marinotti et al. 1990; Moreira-Ferro et al. 1998). This saliva contains substances with anti-haemostatic, anti-inflammatory and immunomodulatory properties (Ribeiro and Francischetti 2003; Andrade et al. 2005). The salivary glands of adult female mosquitoes serve as a dual function, assisting both blood and sugar feeding. Within the anophelines, this salivary cocktail has been exposed through transcriptome analysis of the adult female salivary glands. Some of the most exciting work being done in vector physiology is the discovery and characterization of a large number of proteins and their corresponding genes that are involved in facilitating hematophagy. Classes of proteins, which appear common to all blood-feeding arthropods, include polyphyletic groups of enzymes that prevent coagulation, cause vasodilation and prevent platelet aggregation (Stark and James 1996). Furthermore, proteomic approaches have provided comprehensive lists of individual gene products in the 'sialomes' of various mosquito vectors, including *Ae. aegypti*, *An. gambiae*, *An. stephensi*, *An. darlingi*, *Cx. pipiens quinquefasciatus*, and *An. funestus* (Valenzuela et al. 2003; Francischetti et al. 2002; Calvo et al. 2004; 2006). These studies have revealed an amazing diversity in the recruitment of gene family members to roles in hematophagy as well as a remarkable amount of apparent redundancy in each recognized functional class. Recently, it has been reported that 30 genes expressed in salivary glands of adult *Ae. aegypti* females by hybridization *in situ* patterns was performed on the spatial distribution of gene expression in these organs (Juhn et al. 2011). Distinct spatial accumulation patterns were identified. Genes in proximal-lateral lobe comprise 12 of the 30 genes: alpha-glucosidase, a lysozyme, amylase 1, a salivary chymotrypsin-like gene, two putative vacuolar-type H^+-ATPase subunits, three genes with unknown functions, carbonic anhydrase, gambicin, and a putative serine protease. The last three genes belong to a sub-class of the proximal-lateral lobe group that is transcribed within the anterior-most portion of the proximal-lateral lobes. Next, genes in the distal-lateral lobe group includes 5 of the 30 genes: a member of the D7 family, D7s2, putative 30 kDa allergen-like proteins, 30K a and aegyptin, which also have been designated 30K b; an antigen-5 member, and a putative orthologue of a predicted salivary secreted antigen-5 precursor (AG5-3) from *Cx. quinquefasciatus*. There are five members of the medial lobe group :

sialokinin, a salivary vasodilatory protein, a gene encoding a predicted protein with angiopoietin-like features, a putative C-type lectin, and two genes with unknown functions. The distal-lateral/medial group comprises a serpin, salivary apyrase, D7L1, D7L2, a salivary purine nucleosidase, and two genes with unknown functions. The last group consists of a single transcript of unknown function that accumulates in both proximal and distal-lateral lobes.

In order to facilitate their blood meals, mosquitoes have elaborated a wide range of salivary components that have essential roles in counteracting host haemostatic defences. In addition to these pharmacological activities, salivary components can modulate host immunity at the bite site and induce an immune environment favorable for pathogen transmission. Salivary gland proteins are indicative of incipient understanding of the blood-feeding process.

1.2.5 Symbionts in mosquito midgut

Currently, there is interest in the use of microorganisms as biological control agents of vector-borne diseases. Intestinal microbial flora is a factor believed to contribute to malaria parasite losses at the midgut stage. The mosquito midgut is an immune-competent organ. *Plasmodium* presence in the midgut is known to induce immune responses elsewhere in body, probably due to immune signaling (Rodrigues et al. 2007, 2009). Bacterial microflora (MF) in the midgut lumen may directly interact or adversely affect the parasite. The resident MF in the mosquito midgut contributes towards elicitation of the host's immune response to *Plasmodium* invasion or contribute towards facilitating *Plasmodium* development in mosquitoes. On the other hand, the effect of bacteria on *Plasmodium* development occur indirectly through changes in the physiology of the mosquito host, induction of immune responses that are cross-reactive between bacteria and the parasite.

It is conceivable that mosquito microflora may be manipulated to produce anti-parasitic molecules (Carlson 1996; Conte 1997; Beard et al. 2002; Ramirez et al. 2014). This microflora could then be reintroduced into the insect gut, thus inhibiting parasite development. Paratransgenesis is a newly developed approach proposed as an

anti-*Plasmodium* effector delivery strategy, and genetic modification of symbiotic microorganisms to deliver anti-pathogenic products and thus reduce vector competence.

There are several studies on identification of microflora of field caught (Abalain-Colloc et al. 1987; Hung et al. 1987; Williamson et al. 1996; Straif et al. 1998; Gonzalez-Ceron et al. 2003; Pidiyar et al. 2004; Lindh et al. 2005; Terenius et al. 2008; Rani et al. 2009; Damiani et al. 2010; Zouache et al; 2010, Terenius et al. 2012; Chandel et al. 2013; Ngo et al. 2015) and laboratory reared mosquitoes (Ferguson and Micks 1961; Pumpuni et al. 1996; Straif et al. 1998; Fouda et al. 2001; Gonzalez-Ceron et al. 2003; Rani et al. 2009; Gusmaõ et al. 2010; Ramirez et al. 2014) as are summarized in Table 1.1. An understanding of the microflora diversity of the mosquitoes as fundamental interaction with both the vector and pathogen, is necessary for their potential role in transmission of the parasite and their exploitation in the mosquitoes.

Table 1.1 The diversity of microorganisms associated with mosquitoes using the culture-dependent or culture independent methods.

Insect species	Organs	Microorganisms	References
Ae. aegypti (female adult in lab-reared)	Midgut	*Asaia krungthepensis* *Asaia* sp. *Bacillus* sp. *Bacillus subtilis* *Enterobacter asburiae* *Enterococcus caccae* *Elizabethkingia meningossptica* *Klebsiella pneumoniae* *Pantoea stewartii* *Pichia ohmeri* *Ralstonia paucula* *Serratia marcescens* *Serratia* sp. *Sphingomonas paucimobilis*	Gusmaõ et al. 2010, Terenius et al. 2012
	Gut diverticulum	*Bacillus* sp. *Bacillus cereus* *B. subtilis* *Candida* sp. *C. etchellsii* *Pichia* sp. *Pichia caribbica* *Serratia* sp. *S. marcescens*	Gusmaõ et al. 2007
Ae. aegypti (female adult from field collected)	Midgut	*Acinetobacter* sp. *Asaia* sp. *Bacillus* sp. *Bacillus megaterium*	Zouache et al. 2010
Ae. aegypti (female adult from field collected)	Midgut	*Citrobacter* sp. *Enterobacter* sp. *Pseudomonas* sp. *Serratia* sp. *Staphylococcus saprophyticus*	Zouache et al. 2010
Ae. albopictus (female adult from field collected)	Midgut	*Acinetobacter* sp. *Asaia* sp. *Citrobacter* sp. *Delftia* sp. *Enterobacter* sp. *Herbaspirillum* sp *Pantoea agglomerans* *Pseudomonas* sp.*Pseudomonas putida* *Rickettsia* sp.	Zouache et al. 2010

Table 1.1 (continued)

Insect species	Organs	Microorganisms	References
		Shigella flexneri *Staphylococcus sp.* *Stenotrophomonas maltophilia* *Yokenella regensburgei* *Wolbachia pipientis*	
An. albimanus Wiedemann (female adult in lab-reared)	Midgut	*Acinetobacter* sp. *Flavobacterium* sp. *Pantoea agglomerans* *Pseudomonas cepacia* *Serratia sp.*	Pumpuni et al. 1996
An. albimanus (female adult from field-collected)	Midgut	*Enterobacter amnigenus* *E. cloacae* *Enterobacter sp.* *S. marcescens* *Serratia* sp.	Gonzalez-Ceron et al. 2003
An. funestus (female adult from field-collected)	Midgut	*Anaplasma sp.* *Nocardia corynebacterioides* *Serratia sp.* *S. maltophila*	Lindh et al. 2005
An. darlingi (female adult from field-collected)	Whole mosquitoes	*Aeromanas sp.* *Asaia sp.* *Enterobacter sp.* *Enterobacter hormaechei* *P. putida*	Terenius et al. 2008
An. gambiae (female adult in lab-reared)	Midgut	*Aeromonas hydrophila* *Cedecea lapagei* *Flavobacterium sp.* *Klyvera cryocrescens* *Pantaea agglomerans* *Pseudomonas cepacia* *Pseudomonas gladioli* *Serratia sp.*	Pumpuni et al. 1996
An. gambiae (female adult from field-collected)	Midgut	*A. hydrophila* *Aeromonas sp.* *Achromobacter xylosoiydans* *Anaplasma sp.* *Asaia sp.* *Bacillus cereus* *Bacillus coagulans* *Bacillus mucoides* *Bacillus thuringensis* *C. davisae* *E. coli*	Gonzalez-Ceron et al. 2003, Lindh et al. 2005, Damiani et al. 2010

Table 1.1 (continued)

Insect species	Organs	Microorganisms	References
		Hydrogenophaga pseudoflava *K. pneumonia*	
An. gambiae (female adult from field-collected)	Midgut	*Morganella morgani* *N. corynebacterioides* *P. ananas* *P. aeruginosa* *Pseudomonas putida* *Pseudomonas stutzeri* *S. choleraesuis* *Salmonella enteritidis* *S. marcescens* *Serratia* sp. *S. maltophila*	Gonzalez-Ceron et al. 2003, Lindh et al. 2005, Damiani et al. 2010
An. stephensi (female adult in lab-reared)	Midgut	*A. hydrophila* *C. lapagei* *P. cepacia* *Chryseobacterium meninqosepticum* *Comamonas* sp. *Elizabethkingia meningqosepticum* *Flavobacterium* sp. *P. aeruginosa* *S. marcescens*	Rani et al. 2009
An. stephensi Liston (female adult in lab-reared)	Midgut	*Ewingella americana* *Serratia marcescens* *Staphylococcus* sp.	Pumpuni et al. 1993
An. stephensi (female adult in field-collected)	Midgut	*Acinetobacter* sp. *Acinetobacter hemolytic* *Acinetobacter radioresistens* *C. meninqosepticum* *Chryseobacter indologenes* *Citrobacter freundii*	Rani et al. 2009
An. stephensi (female adult in field-collected)	Midgut	*Comamonas sp.* *E. meningqosepticum* *Enterobacter sp.* *Enterobacter cloacae* *Enterobacter sakazaki* *Enterobacter hermani* *Pseudomonas aeruginosa* *P. putida* *Pseudomonas synxantha* *Pseudomonas sp.* *S. marcescens* *Serratia nematodiphila*	Rani et al. 2009

Table 1.1 (continued)

Insect species	Organs	Microorganisms	References
An. barbumbrosus (adult in wild-collected)	Midgut	*Acinetobacter* sp. *Brachybacterium* *Chryseobacterium* *Enhydrobacter* *Knoellia* sp. *Leucobacter* sp. *Microbacterium* sp. *Staphylococcus* sp. *Xanthomonas* sp. *Yersinia* sp.	Ngo et al. 2015
An. crawfordi	Midgut	*Acinetobacter* sp. *Brachybacterium* *Brevibacterium* *Enhydrobacter* *Enterococcus* sp. *Klebsiella* sp. *Pseudomonas* sp. *Serratia* sp. *Staphylococcus* sp. *Thorsellia* sp.	Ngo et al. 2015
An. gigas	Midgut	*Acinetobacter* sp. *Bacillus* *Bartonella* *Staphylococcus* *Thorsellia* sp.	Ngo et al. 2015
An. maculatus	Midgut	*Janibacter* sp. *Staphylococcus*	Ngo et al. 2015
Cx. quinquefasciatus (female adult in field-collected)	Midgut	*Aeromonas jandaei* *Aeromonas hydrophila* *Acinetobacter sp.* *Acinetobacter baumannii* *Acinetobacter beijerinckii* *Acinetobacter junii* *Acinetobacter calcoaceticus* *Bacillus anthracis* *Bacillus cereus* *Bacillus thuringiensis* *Citrobacter braakii* *Delftia lacustris* *Enterobacter cloacae* *Enterobacter faecalis* *Enterococcus seriolicida* *Lactococcus garvieae*	Pidiyar et al. 2004, Gouveia et al. 2008, Terenius et al. 2012, Chandel et al. 2013

Table 1.1 (continued)

Insect species	Organs	Microorganisms	References
		Janibacter melonis *Klebsiella oxytoca* *K. pneumonia* *Lactococcus lactis* *Lysinibacillus macrolides* *Microbacterium arborescens* *Microbacterium oxydans* *Pantoea anthophila* *Pantoea agglomerans* *Pseudomonas* *P. aeruginosa* *S. marcescens* *Staphylococcus epidermidis* *Staphylococcus gallinarum* *S.maltophila* *Vogococcus fluvialis*	
Culex fatigans (female adult in lab-reared	Midgut	*Lactobacillus* sp. *Alcaligenes* sp. *Pseudomonas* sp.	Ferguson and Micks 1961
Cx. pipiens (female adult from field-collected)	Midgut	*Aeromonas* sp. *Acientobacter* sp. *Comamonas* sp. *Flavobacterium* sp. *Klebsiella* sp. *Pseudomonas* sp. *Sphingobacterium* sp	Demaio et al. 1996

1.2.6 *Anopheles dissidens* mosquito

An. dissidens (*An. barbirostris* species A1) mosquito is a member of the Barbirostris Complex. The Barbirostris Complex mosquitoes are widely distributed from Indian through mainland Southeast Asia and southward through Indonesia to Sulawesi (Reid 1968) and considered as important vectors of malaria and Brugian filariasis in Sulawesi, Flores, and Timor (Atmosoedjono et al. 1977; Reid et al. 1979).

In Thailand, *An. dissidens* mosquito is a low potential vector for *Plasmodium vivax* with approximately 9.09% sporozoite rates in comparison to the 85.71-92.31% sporozoite rates recovered from the susceptible mosquito, *An. cracens* (Thongsahuan et al. 2011). The mosquito occurs in hilly country and the female is mainly zoophilic. Saeung et al. (2007) have reported that seventeen isolines of *An. barbirostris* derived from animal-biting female mosquitoes showing three karyotypic forms: Form A (X_2, Y_1), Form B (X_1, X_3, Y_2), and Form C (X_2, Y_3). Furthermore, similar studies have shown that *An. barbirostris* s.l. is a cryptic species consisting of at least four sibling species, i.e., A1, A2, A3 (Saeung et al. 2008) and A4 (Suwannamit et al. 2009). The *An. barbirostris* species A1 (Forms A, B, and D), A2, A3, and A4 and *Anopheles campestris*-like (Forms B and E) salivary gland proteins were analyzed (Jariyapan et al. 2010). At least eight major and several minor protein bands were detected in the glands of each species, of which each morphological region contained different major proteins. Recently, Taai and Harbach (2015) have reported three new species of the Barbirostris Complex, *An. dissidens*, *Anopheles saeungae* (formerly *An. barbirostris* species A2), and *Anopheles wejchoochotei* (formerly *An. campestris*-like). The complex is compared with *Anopheles barbirostris* van der Wulp and *Anopheles campestris* Reid based on specimens of molecularly identified progeny broods.

1.3 Purpose of This Study

1.3.1 To investigate morphological and protein changes in the midgut of female *An. dissidens* during adult development and blood feeding.

1.3.2 To investigate morphological and protein changes in the salivary glands of female *An. dissidens* during adult development and blood feeding.

1.3.3 To investigate bacterial diversity in the midgut of sugar-fed *An. dissidens.*

1.4 Usefulness of This Study

Intensive knowledge on morphological changes in the midgut and salivary glands of *An. dissidens* could contribute to a better understanding of the physiological processes that appear during adult development and blood feeding. Also, information on proteins would be an initial step for further study on proteome of the mosquito midgut and salivary glands and all bacteria isolated from this study would be evaluated further for their suitability as a paratransgenic candidate.