EXECUTIVE SUMMARY

Anopheline mosquitoes are the exclusive vectors of human malaria. Salivary glands are of interest in anopheline mosquitoes because transmission of malaria to vertebrate hosts depends on the ability of *Plasmodium* sporozoites to invade the salivary glands of female vector mosquitoes. Thailand is an endemic area for malaria. A number of sibling species complexes of Anopheline mosquitoes are the human malaria vectors, for example, *Anopheles dirus* and *Anopheles minimus*. Recently, *An. barbirostris* complex, i.e., *An. campestris*-like (Chiang Mai strain), *An. barbirostris* species A1, A2, A3, and A4 of Thai populations were discovered and tested for susceptibility to indigenous strains of *Plasmodium vivax*. The results showed that *An. campestris*-like (Form B and E, Chiang Mai strain) was a high potential vector whereas *An. barbirostris* species A1, A2, and A3 were low potential vectors. For *An. barbirostris* species A4, it was a refractory vector for *P. vivax*. As all are morphologically indistinguishable, incorrect identification of individual members in the complex may result in failure to distinguish between a vector and non-vector species, and lead to the complication and/or unsuccessful formation of vector control strategies. Although the ITS2 sequence of species A4 (1,676 bp) can be used to distinguish species A1 (1,861 bp), A2 (1,717 bp), and A3 (1,070 bp), it is slightly different from *An. campestris*-like (1,651 bp). Thus, it is difficult to differentiate the size of the ITS2-PCR products between species A4 and *An. campestris*-like on a 0.8% agarose gel. In sandflies, electrophoretic profiles of salivary proteins are able to distinguish phlebotomine species. Also, electrophoresis of salivary heme proteins could be used to identify morphologically similar *Rhodnius* species. In addition, salivary gland proteins and/or genes are proposed to be a useful tool for further analysis of the *Anopheles darlingi* taxonomic status. Therefore, in this study, the salivary glands of female mosquitoes of the five sibling species in the *An. barbirostris* complex were analyzed by SDS-PAGE, 2-DE, and nanoLC-MS.

SDS-PAGE analysis showed that 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. The protein profiles distinguished the five sibling species. The variability in major proteins among species was observed in the 40-48 kilodalton (kDa), 32-37 kDa and 10-18 kDa ranges. No difference in protein profiles was found in different cytogenetic forms. Polymorphism of the protein profiles within species was only noted in species A4. The lowest major protein (marker) band of each species showed remarkably different relative mobility on

SDS-polyacrylamide gels. NanoLC-MS analysis revealed that the marker protein of some species matched with a protein involving in blood feeding, gSG6, of *An. gambiae* and *An. freeborni*. Two-dimensional gel electrophoresis analysis revealed that at least 14 major and several minor protein spots were detected in the female salivary glands of each species. However, less than half of numbers of the major protein spots of each species were identified by nanoLC-MS. Four protein families were commonly found in the salivary glands of the five sibling species including apyrase/5'-nucleotidase, anti-platelet (GE-rich/30 kDa), D7/D7-related, and gSG6.

Detail analyses on the expression of salivary gland proteins in mosquitoes aged varying from 0 to 60 hours post emergence and the differential distribution of salivary components within the glands of female mosquitoes were performed in *An. barbirostris* species A2. Two-dimensional gel electrophoresis revealed approximately 75 well-resolved spots on the reference gel. Most of the protein spots displayed relative molecular masses from 14 to 85 kilodaltons and isoelectric points ranging from 3.9 to 10. The proteome profiles of *An. barbirostris* species A2 female salivary glands were affected by ageing. The typical electrophoretic pattern of the female salivary glands was reached in 48 hours post emergence suggesting the maturation of salivary glands and saliva contents for blood feeding. Proteins involved in blood feeding, i.e., putative 5' nucleotidase/apyrase, anti-platelet protein, long form D7 salivary protein, D7-related 1 protein, and gSG6. Salivary proteins start to accumulate from emergence and gradually increase becoming predominant within 48 hours. There are different salivary components expressed within each region of the female glands. The blood feeding proteins were detected in the distal-lateral lobes and/or medial lobes.

In addition, *An. campestris*-like salivary gland proteins were determined and analyzed. The total amount of salivary gland proteins in the mosquitoes aged 3-5 days was approximately 0.1 ± 0.05 μg/male and 1.38 ± 0.01 μg/female. Two-dimensional gel electrophoresis showed approximately 20 major and several minor protein spots displaying relative molecular masses from 10-72 kilodaltons with electric points ranging from 3.9-10. At least 15 glycoproteins were detected in the female glands. Similar electrophoretic protein profiles were detected comparing the male and proximal-lateral lobes of the female glands, suggesting that these lobes are responsible for sugar feeding. Blood feeding proteins, i.e., putative 5'-nucleotidase/apyrase, anti-platelet protein, long form D7 salivary protein, D7-related 1 protein, and gSG6, were detected in

the distal-lateral lobes (DL) and/or medial lobes (ML) of the female glands. The major spots related to housekeeping proteins from other arthropod species including *Culex quinquefasciatus* serine/threonine-protein kinase rio3 expressed in both male and female glands, *Ixodes scapularis* putative sil1 expressed in DL and ML, and *I. scapularis* putative cyclophilin A expressed in DL.

Proteins detected and/or identified by these approaches could be tested in strategies developed to control pathogen and disease transmission. Moreover, the information of the 2D maps of the female salivary glands might be useful for construction of an additional tool to distinguish species members in the complex. Furthermore, because of differences in their roles in the transmission of malaria and filariasis, description of the salivary proteomes and transcriptomes of the *An. barbirostris* complex is required. Comparative analysis of the proteomes/transcriptomes of the sibling species may supply better tools for determination of phylogeny of closely related species, population structure and speciation processes, and ultimately, identification of genes related to vectorial capacity and host preference.