CHAPTER 2

LEITERATURE REVIEWS

Malaria remains the most prevalent and devastating insect-borne parasitic disease in humans, with 255 million cases reported worldwide yearly. However, the number of deaths due to malaria is estimated to have decreased from 985,000 in 2000 to 781,000 in 2009 (WHO 2010). In Thailand, four species of human malaria parasites are found; the most common being *P. vivax* (50.74%) and *Plasmodium falciparum* (48.61%), while *Plasmodium malariae* (0.20%) and *Plasmodium ovale* (one case reported from Chiang Mai Province in 1996) are rare, and 0.45% are mixed infections. The disease is generally limited to rural communities living in and near forested regions, mountains and foothills, particularly those people residing in newly opened land settlements of semi-forested areas, where they earn their living by growing agricultural crops. Regions near and along the borders with neighboring countries, i.e., Cambodia, Laos, Myanmar and Malaysia, are also affected.

Anopheline mosquitoes are the exclusive vectors of human malaria. Salivary glands are of interest in the 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. The female salivary glands produce a wide array of secreted compounds that are delivered with the saliva and help blood-feeding by affecting the host hemostatic response. In addition, the saliva is the vehicle that carries pathogens and may also enhance or facilitate infectivity during the blood meal (Ribeiro and Francischetti 2003). Analyses of salivary gland proteins (sialomes) and/or genes (transcriptomes) of anopheline mosquitoes were performed in *An. gambiae* (Arca et al. 1999a, b, 2005; Dana et al. 2005; Kalume et al. 2005a, b; Francischetti et al. 2002; Lanfrancotti et al. 2002), *An. stephensi* (Valenzuela et al. 2003), *An. darlingi* (Calvo et al. 2004), *An. funestus* (Calvo et al. 2007) and *An. dirus* B [= *cracens* (Sallum et al. 2005a)] (Jariyapan et al. unpublished data). Calvo et al (2004) compared *An. darlingi* (subgenus *Nyssorhynchus*) and *An. gambiae* (subgenus *Cellia*) salivary gland genes belonging to the secreted and housekeeping categories. They concluded that the salivary gland genes encoding

secreted products are rapidly evolving in comparison with the housekeeping genes of these species. Valenzuela et al (2003) found similar results when the salivary gland transcriptomes of *An. stephensi* and *An. gambiae* were compared. These two species belong to the same subgenus (*Cellia*) and when compared showed 93% of identity for gene products of the housekeeping group whereas the salivary proteins are only 62% identical. These results support the idea that secreted genes may be good markers for assessing phylogeny among closely related species, as has been demonstrated with triatomine bugs using the salivary hemeproteins (Soares et al. 1998, 2000). In *An. darlingi,* previous studies based on behavioural (patterns of biting activity), morphological (body size and polytene chromosome patterns) and molecular (allozymes and ITS2 sequences) differences among geographically distinct populations have indicated the possibility that *An. darlingi* is a complex of closely related species (Lounibos and Conn 2000). Salivary gland proteins and/or genes are proposed to be a useful tool for further analysis of the *An. darlingi* taxonomic status (Calvo et al. 2004).

In Thailand, there are at least 18 anopheline species playing an important role as primary, secondary and suspected vectors of malaria transmission. The primary vectors are *Anopheles dirus* complex [*Anopheles dirus s.s.* (species A), *Anopheles baimaii* (species D)], *Anopheles minimus* complex [*An. minimus s.l.* (species A)] and *Anopheles maculatus* complex [*Anopheles maculatus s.s.* (species B)], while *Anopheles aconitus* and *Anopheles sundaicus* complex [species A = *epiroticus* (Linton et al. 2005)] are considered as secondary vectors (Gould et al. 1967; Scanlon et al. 1968; Harrison 1980; Rosenberg et al. 1990; Rattanarithikul et al. 1996; Subbarao 1998; Sallum et al. 2005a, b). Subsequently, *Anopheles pseudowillmori*, a member species of the *maculatus* complex, has been incriminated as a secondary vector (Green et al. 1991). Recently, *An. campestris* (identification was based only on the summation of seta 2-VI branches of pupal skins) was incriminated as a potentially natural vector of *P. vivax* in Pa Rai subdistrict of Aranyaprathet district, Sa Kaeo province (Apiwathnasorn et al. 2002). The remaining 11 species, i.e., *Anopheles annularis*, *Anopheles karwari*, *Anopheles kochi*, *Anopheles nigerrimus*, *Anopheles nivipes*, *Anopheles peditaeniatus*, *Anopheles philippinensis*, *Anopheles sawadwongporni*, *Anopheles sinensis*, *Anopheles tessellates*, and *Anopheles vagus* are suspected vectors, since they were found positive by an ELISA method for oocysts in the midgut and/or circumsporozoite

antigens (Baker et al. 1987; Harbach et al. 1987; Gingrich et al. 1990; Frances et al. 1996; Rattanarithikul et al. 1996).

The anthropophilic *An. barbirostris/campestris* group has been reported firstly as a probable vector of malaria in Pa Rai subdistrict, Aranyaprathet district, Sa Kaeo province (Limrat et al. 2001). The increase in population, high biting density, anthropophilicity, high susceptibility to *P. vivax* and detection of circumsporozoite protein (Pv 247) (Somboon et al. 1994; Frances et al. 1996; Limrat et al. 2001; Apiwathnasorn et al. 2002), have caused this species group to be considered as a possible, important vector corresponding to the increase of *P. vivax* prevalence in Thailand (Sattabongkot et al. 2004). Based on the identification of pupal skins, Apiwathnasorn et al (2002) subsequently incriminated *An. campestris* as a potentially natural vector of *P. vivax* in this locality, although investigations into the summation of branches of seta 2-VI of 500 pupal skins, derived from 50 iso-female lines, revealed 94.4% of 17-58 branches (*An. campestris*), 14.8% of 17-18 branches (overlapping), and 5.6% of < 18 branches *(An. barbirostris*). The crucial question as to whether the 14.8% overlapping range is *An. campestris*, *An. campestris*-like or *An. barbirostris* is still ambiguous. Exact species identification by using metaphase karyotypes (Baimai et al. 1995), which have so far been proven as the only reliably diagnostic tools, should be intensively carried out prior to drawing the final conclusions.

Little is known about the *An. barbirostris/campestris* group from the population genetic point of view, or its exact role as a vector of malaria in many localities of Thailand. Reproductive isolation between two strains of *An. barbirostris* from Chon Buri and Chumporn province were first demonstrated by Choochote et al (1983). The results indicated that these two strains exhibit a possible presence of a species complex. Subsequently, three karyotypic forms of *An. barbirostris* (Form A: X_2, X_3, Y_1; Form B: X_1, X_2, X_3, Y_2; and Form C: X_2, X_3, Y_3) have been reported throughout Thailand, whereas Form D (X_2, Y_4) has been reported from Java, Indonesia by Baimai et al (1995). Recently, an additional, new karyotypic form of *An. campestris*-like [based on the summation of seta 2-VI branches of pupal skins 22.25 (18-30)], designated Form E (X_2, Y_5), was obtained from Chiang Mai province, northern Thailand (Saeung et al. 2007). Its metaphase karyotype, particularly the Y-chromosome is markedly distinct from the four forms of *An. barbirostris* and *An. campestris*, i.e., Y_1 is subtelocentric, Y_2 is large submetacentric, Y_3 is large submetacentric or metacentric, and Y_4 is medium metacentric in *An. barbirostris* Form A, B, C,

and D, respectively; Y_5 is small metacentric in *An. campestris*-like Form E; and Y is telocentric in typical *An. campestris*. In addition, investigations of post-mating barriers by hybridization between sympatric *An. campestris*-like Form E and *An. barbirostris* Form B revealed strong genetical incompatibility, providing low embryonation and hatchability rates, retained stage and inviability of hatched larvae. Another interesting point is that the crossing between two allopatric *An. barbirostris* Form A strains from Chiang Mai and Phetchaburi province demonstrated extensive reproductive isolations, providing low viability of F_1-hybrid larvae with asynaptic salivary gland polytene chromosomes, sex distortion of adults, abnormal development of ovarian follicles of F_1-hybrid females, and atrophy of the accessory glands and testes of F_1-hybrid males. Choochote et al (personal communication) have investigated karyotypic variations of the *An. barbirostris* complex in the systematic direction and found that the *An. campestris*-like Form E is susceptible in a high level to *P. vivax* in laboratory but the *An. barbirostris* Form A is not a malaria vector. This result corresponds to the report of Apiwathnasorn et al (2002).

Despite their importance as malaria vectors, no protein/nucleotide sequence of the *An. barbirostris* complex salivary glands is available in the NCBI database. The comparative analysis of the proteomes and/or transcriptomes of the mosquito members in this complex that having distinct primary host [human (vector) or other animals (non-vector)], may supply better tools for determination of phylogeny of closely related species, population structure and speciation processes, and ultimately identify genes related to vectorial capacity and host preference. All of this information is likely to be useful for the improvement of existing and development of novel transmission-reduction malaria control strategies.