MALARIA VECTORS IN THE GREATER MEKONG SUBREGION: OVERVIEW OF MALARIA VECTORS AND REMAINING CHALLENGES

Abstract. Malaria transmission in the Greater Mekong Subregion depends on, among other factors, vector behavior and ecology, and the degree of contact between humans and the Anopheles mosquitoes. This chapter will review and update knowledge presented in the 2003 Mekong Malaria monograph for planning and implementing evidencebased vector control programs. Collation of 150 publications and reports showed that the highest number of vector species reported included An. minimus Theobald complex (26.74%), An. dirus Peyton and Harrison (14.26%), An. sundaicus (Rodenwaldt) (5.65%), An. sinensis Wiedemann (4.29%), An. maculatus Theobald (4.23%), An. philippinensis Ludlow (1.62%), An. annularis Van der Wulp (0.37%), An. campestris Reid (0.25%), and An. lesteri Baisas and Hu (=anthropophagus) (0.045%). Other Anopheles species accounted for 44.44%. Anopheles dirus was incriminated as a vector of Plasmodium knowlesi malaria in Viet Nam for the first time, but remained a suspected simian vector in other countries in the region. Well-designed trials of innovative strategies in intractable and difficult situations are needed, including a better understanding of the various causal relations and interactions between physiology, environment, and vector bionomics. While current front-line vector control interventions have contributed significantly to a worldwide decrease of malaria, indoor residual spraying and insecticide-treated bednets/ long lasting insecticidal bednets have had variable impact on exophilic/exophagic and/or early biting vectors. As vectors' responses to control measures vary in different areas, entomological studies on the efficacy of insecticide-treated bednets and other innovative control tools to ensure that strategies are tailored to local circumstances. Given that current tools are insufficient to break transmission cycles, more strategic investments into research on outdoor transmission, monitoring of insecticide resistance, vector species identities, vector mapping, target profiles of new control technologies and delivery systems are required.

Keywords: *Anopheles dirus, minimus, sinensis, sundaicus* complexes, vector bionomics, personal protection, control, elimination, Mekong, Myanmar, Thailand, Yunnan, Lao PDR, Cambodia, Viet Nam

INTRODUCTION

This review includes malaria vectors in the six Greater Mekong Subregion (GMS) countries which have an estimated combined population at risk of 92 million (Chapter 2). Vector borne diseases have a high impact on morbidity in these countries and of these diseases malaria causes the most deaths in remote and border areas. The GMS countries have a reputation for the high biodiversity of vector species, a high number of *Anopheles* species complexes and suspected species complexes, tremendous spatial heterogeneity in distribution patterns and behavioral plasticity both between and within species which additively increases the entomological complexity in the Asia-Pacific region (Cui *et al*, 2012; Singhasivanon *et al*, 2003; Sinka *et al*, 2011; WHO, 2007). The divergent ecological systems in GMS provide diverse breeding sites for multiple mosquito vector species with distinct preferences for different localities in a region such as forest edges, foothills or agricultural fields (Cui *et al*, 2012). This influences the variability of malaria transmission and spatial distribution patterns among and between countries and is a major contributing factor to the continuing disease burden in the subregion (Chapter 1).

The control of malaria relies on knowledge of the vector species involved. Although the dominant *Anopheles* vector species have been identified in many areas (Sinka *et al*, 2011), there is a need for more information and clarification of the taxonomy and the corresponding bionomics of many of these species and on the role of other *Anopheles* in the region in malaria transmission. Information on the distribution of vectors is patchy, and in some cases out of date, given the rapid environmental changes taking place in the Asia-Pacific region, particularly deforestation (Chapter 1; AusAID, 2012).

This chapter thus aims to: 1) identify the relative medical importance of the dominant, secondary and suspected malaria vectors in each country by synthesizing recent scientific literature from 2003 to 2012; 2) update the distributions of the *Anopheles* vectors using literature records and other information; 3) provide an overview of intra- and inter-species variation of biology and behavior; and 4) identify important knowledge gaps in vector biology and disease transmission in order to develop more cost-effective vector control and personal protection measures.

MATERIALS AND METHODS

Reports were identified through searches of PubMed for those published from January 1, 2003 to October 31, 2012, with the terms "malaria", "*Anopheles*", "vectors" combined with specific country names. The analysis was restricted to English language articles. The WHO regional websites, the World Malaria Report, the Mekong Malaria Forum, Google Scholar, and the Walter Reed Biosystematics Research Unit/US Armed Forces Pest Management Board Literature Retrieval System databases and related published articles were searched for additional studies of malaria vectors from included countries. We identified national vector control and prevention policies for malaria on the websites of each country's Ministry of Health.

Related data in Table 1 were also retrieved from numerous publications for a) Cambodia: Van Bortel *et al,* 2002; Sovannaroth, 2005; b) China: Liu *et al,* 2010; Rueda *et al,* 2005a,

2007; Wen *et al*, 2006; c) Lao PDR: Vythilingam *et al*, 2003, 2005a,b, Sidavong *et al*, 2004; CMPE, 2009; d) Myanmar: Oo *et al*, 2002, 2006; e) Thailand: Hu *et al*, 2003; Jariyapan *et al*, 2005; Junkum *et al*, 2005, 2007; Somboon *et al*, 2005a,b, 2009; Rongnoparut *et al*, 2006; Saeung *et al*, 2007, 2008; Walton *et al*, 2007; Suwannamit *et al*, 2009; Thongsahuan *et al*, 2009, 2011; Paredes-Esquivel *et al*, 2009; Phasomkusolsil and Soonwera, 2010; Choochote, 2011; Otsuka, 2011; Intirach *et al*, 2012; Kongmee *et al*, 2012; f) Viet Nam: Verhaeghen *et al*, 2010; Ohba *et al*, 2011; Van Bortel *et al*, 2001, 2009, 2010.

RESULTS

A total of 150 publications reporting on >806,446 *Anopheles* mosquitoes from six GMS countries were identified between 2003 and 2012 (Table 1). Overall, the vector species most often reported included *An. minimus* Theobald complex (26.74%), *An. dirus* Peyton and Harrison (14.26%), *An. sundaicus* (Rodenwaldt) (5.65%), *An. sinensis* Wiedemann (4.29%), *An. maculatus* Theobald (4.23%), *An. philippinensis* Ludlow (1.62%), *An. annularis* Van der Wulp (0.37%), *An. campestris* Reid (0.25%), *An. lesteri* Baisas and Hu (*=anthropophagus*) (0.045%), *An. jeyporiensis* (0.031%) and other anophelines (44.44%). In the full MALVECASIA dataset described in Van Bortel *et al* (2008), the frequency of *Anopheles* vectors collected was as follows: *An. epiroticus* Linton and Harbach (19.99%), *An. minimus* (15.59%), *An. annularis* (9.97%), *An. maculatus* (7.15%), *An. dirus* (5.43%) and *An. jeyporiensis* James (4.42%) (data presented in Obsomer, 2010).

The data from Myanmar is based on extensive collections by Oo *et al* (2004) and Sinka *et al* (2011) from 1,791 mappable locations which are considered the greatest number of sites among the 31 countries with a reported presence of one or more of the 19 dominant vector species (DVS).

The main methods of sampling *Anopheles* mosquitoes included adult collections (62.4%; 244/391), insectary colonies (12.53%; 49/391) and larval collections (10.23%; 40/391), while a small proportion used adult and larval collections combined (5.4%; 21/391) or did not specify the numbers of specimens used (6.14%; 24/391) (Table 1). In general, the number of collection methods used was slightly greater in *An. dirus* (24.55%; 96/391) than *An. minimus* (23.52%; 92/391), followed by *An. maculatus* (8.44%; 33/391), *An. sinensis* (5.88%; 23/391), *An. sundaicus* (5.63%; 22/391) and *An. campestris* (2.56%; 10/391) (Fig 1).

Adult collections were made primarily from human landing catches (HLC) (30.67%), animal baits (24.37%), larval collections (12.6%), indoor resting and insectary colonies (9.24% each), light trap collections (6.30%), and human-baited net traps (2.10%). Some methods were also used in combination, *eg* human landing/animal bait /indoor resting/larval surveys/





Fig 1–Summary of Anopheles species collection methods between 2002 and 2012.

light trap collections. Results of concurrent CDC light traps used for field entomological monitoring were variable, and they accounted for a total of 98% of indoor *An. dirus* s.l.and 46.7% of indoor *An. minimus* s.l. in Lao PDR compared to zero *An. dirus* s.l. collections and 28.6% of indoor *An. minimus* s.l. in Viet Nam (Pongvongsa *et al*, 2012). These results provide a useful and direct estimate of human biting rate although they do not specifically capture mosquitoes engaged in host-seeking.

Several studies have compared human landing collections (HLCs) with various light-trap collection (LTC) designs for estimating relative sampling efficiencies. The results show widely varying relative sampling efficiencies from the expected 1:1 LTC:HLC. A comparative evaluation of light-trap catches, electric motor mosquito catches and human biting catches showed that light traps collected 1.52 times (range: 1.35-1.71) the number of *An. sinensis* compared to human biting catches (r=0.82, p<0.01) (Wang *et al*, 2012a). The study was carried out in the Three Gorges Reservoir, China in four representative villages for 256 nights over 16 months, with a final comparison between 256 nights for LTC and 512 person-nights for HLCs (Wang *et al*, 2012b). Over a 24-month period, CDC light traps baited with dry ice collected 40 times more *An. sundaicus* compared to gravid traps with fermented hay at five areas in Thailand

Table 1	The number and proportion of dominant Anopheles species found in studies betw	for individual species only where those species are officially recognized as vector
nent 1)	20	13

nber and proportion of dominant <i>Anopheles</i> species found in studies between 2003 and 2012 ^a . Numbers are presente
idual species only where those species are officially recognized as vectors, see World Malaria Report 2011 and Count
Profiles (WHO, 2011a).

Country					Numbers	of Anoph€	eles species	and proportion				
(no. of references)	dirus	minimus	sundaicus	maculatus	sinensis	lesteri	annularis	philippinensis	campestris	jeyporiensis	Other Anopheles	Total
Cambodia(13)	5,668	7,186	473	2,822							9,706	25,855
China (24)	06	712			33,977	361					696	35,836
Lao PDR (13)	3,843	10,378		1,464						249	10,518	26,452
Myanmar (6)	3,292	10,530	2,658				2,965				83,735	103,180
Thailand (73)	51,516	143,799	3,595	29,189				12,790	1,956		132,470	375,315
Viet Nam (23)	48,414	38,909	37,994								114,491	239,808
Total	112,823	200,284	40,606	33,475	33,977	361	2,965	12,790	1,956	249	351,602	806,446
%	14.26	26.74	5.65	4.23	4.29	0.045	0.37	1.62	0.25	0.031	44.44	
^a Not included are	e unspecifie	d collections	from southern	China, Thaila	and and no	rthern Viet	: Nam where	1,300 of 5,000 A	<i>Inopheles</i> spe	cimens were sp	ecies of the	Myzomyia
Series of the sui	bgenus Cell	lia, and appro	oximately 600	specimens w	vere morph	nologically	identified to	the An. minimus	s complex (Ch	nen <i>et al</i> , 2011)		

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(Changbunjong *et al*, 2012). However, evaluation of candidate traps in Thailand showed that landing/biting (L/B) collections were far more effective at collecting the primary Thai vectors than any of the other trap methods such as CDC light traps, American Biophysics Corporation (ABC) standard light trap baited with dry ice and octenol, the ABC counterflow geometry trap with dry ice and octenol, the ABC mosquito magnet trap with octenol, and the Nicosia and Reinhardt Company Mosquito Attractor Device (Sithiprasasna *et al*, 2004). The L/B method caught 93% of all *An. dirus*, 81% of all *An. maculatus*, 80% of all *An. sawadwongporni*, and 72% of all *An. minimus* compared to the other collection methods. These comparative studies are relevant to entomological monitoring activities in the GMS as the World Health Organization recommends avoiding HLCs unless absolutely essential, especially if safer techniques are available that can provide proxy estimates of human biting rates (WHO, 2003; Overgaard *et al*, 2012).

1. Biosystematics, distribution, identification of sibling species and phylogeny

The accurate identification of any *Anopheles* vector involved in malarial parasite transmission is essential to fine tune interventions and maximize cost-effectiveness. Any misidentification of vectors could result in the unnecessary use and misdirection of limited resources for control interventions in the GMS where the close relationship and sympatric distributions (Co-existence of two species or populations in the same geographic area and thus regularly encounter one another.) of many of the species are fairly common. Furthermore, any drastic changes in the environment, urbanization, deforestation, newly implemented irrigation programs and expanding agricultural development can rapidly affect the composition of the local mosquito fauna and subsequently influence the management and control methods required (Sinka *et al*, 2011).

Table 2 shows a partial list of species and geographical distribution of common *Anopheles* vector groups in the GMS. We adopted the arrangement of taxa (including groups, subgroups and complexes) as that proposed by Harbach (2004) and Rattanarithikul *et al* (2005, 2006), except for those new or temporary ones. We also used the following terms: *sensu stricto* (*s.s.*) to mean 'in the strict sense', *ie* the exact species, and *sensu lato* (*s.l.*) meaning 'in the broad sense', *ie* any or all members of the species complex. In this paper we report about 52 genetic forms in the GMS, and about 39 forms remain unnamed and require further study to determine if they are distinct species or intra-species genetic polymorphs (Table 3). Brief notes are provided on the taxonomy, bionomics, distribution and vectorial capacity of those *Anopheles* groups/subgroups of medically important species in the GMS. A more comprehensive review of all groups of *Anopheles* (both vector and non-vector species) in the GMS will be provided in a separate paper.

1.1 Dirus complex

The Dirus complex belongs to the Leucosphyrus Subgroup under the Leucosphyrus Group of the Neomyzomyia Series, Anopheles (Cellia) (Table 2). The Leucosphyrus Group includes 20 species in Asia, of which 10 species are found in the GMS. Within the Leucosphyrus Group, the Dirus complex is the most common collection of forest and forest-fringe inhabiting mosquitoes from the GMS. Seven species of the Dirus complex are An. baimaii Sallum and Peyton, An. cracens Sallum and Peyton, An. dirus, An. elegans (James), An. nemophilous Peyton, An. scanloni Sallum and Peyton, and An. takasagoensis Morishita. Compared to most species in the Leucosphyrus Group that have infrequent contact with humans, two members (An. dirus and An. baimaii) are regarded as extremely efficient malaria vectors because of their highly endophagic and anthropophilic behavior. Five species within the Dirus complex are present in the GMS, including in Thailand (5 species), China (2 species), and one species each in Viet Nam, Cambodia, Myanmar and Lao PDR (Sallum et al, 2005a, b, 2007). The remaining two complex members are restricted to southwestern India (An. elegans [James]) and Taiwan (An. takasagoensis) (Sallum et al, 2005a). In Thailand, An. dirus s.l. is currently known to comprise five species, namely An. dirus s.s. (= An. dirus sp A), An. cracens (= An. dirus sp. B), An. scanloni (= An. dirus sp C), and An. baimaii (= An. dirus sp D) and An. nemophilous (= An. dirus F). Because of taxonomic confusions, they were all previously regarded as malaria vectors with sporozoite rates of up to 10% (Peyton, 1989; Sallum et al, 2005a, b). Although An. baimaii and An. dirus are considered to be among the more important primary malaria vectors in Thailand (Rattanarithikul et al, 2006; Manguin et al, 2008a, b, 2010), both species may also be the primary vectors in the other GMS countries, depending on their specific distribution. In the GMS, An. dirus occurs mainly in the eastern part of Thailand through Lao PDR, Cambodia and Viet Nam (south of the Red River), while An. bamaii spreads predominantly to the west of Thailand through Myanmar and southern China (Hunan) (Sallum et al, 2005a, b; Obsomer et al, 2007).

Fig 2A shows the hypothesized range of the *An. dirus* complex based on expert opinion and known species occurrence records (Hay *et al*, 2010). Detailed distribution of the sibling species depicted on a vegetation background is provided in Obsomer *et al* (2007).

Other members of the Leucosphyrus Group in the GMS include two species of the Leucosphyrus complex (in Leucosphyrus Subgroup), namely *An. latens* Sallum and Peyton and *An. introlatus* Colless. Table 2 includes the distribution of the species in the Dirus complex and other members of the Leucosphyrus Group.

Morphological identification in the Dirus complex is difficult due to overlapping characters and *"the variability of their elaborate ornamentations"* as highlighted in a recent revisionary

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Internal classification, partial list of species and geographical distribution of common Anopheles vector groups in the GMS and

			elsewh	here.			
Subgenus/ Series/ Group	Subgroup	Complex	Species ^a	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)	Reference ^c
<i>Anopheles/</i> Myzorhynchus/ Barbirostris	Barbirostris	Barbirostris	<i>An. barbirostris</i> Van der Wulp s.I.	5	Cambodia, China, Lao PDR, Myammar, Thailand, Viet Nam	Bangladesh, India, Indonesia, Pakistan, Sri Lanka	4, 12
		,	<i>An. campestris</i> Reid	C (D, F)	Cambodia, China, Thailand, Viet Nam	Malaysia	4, 12
		·	An. donaldi Reid	(D, F)	Lao PDR, Thailand	Indonesia, Malaysia	4, 12
			<i>An. hodgkini</i> Reid	U	Thailand	Australia	4, 12
			An. pollicaris Reid	Л	Thailand	Malaysia	4, 12
Anopheles/	Lesteri	Crawfordi	An. crawfordi Reid s.l.	Л	Cambodia, China, Theilend Mint	India, Indonesia, Malaxia	4, 12
Myzornyncnus/ Hyrcanus					i naliano, viet Nam	Malaysia	
		,	<i>An. belenra</i> e Rueda	(D)	China	North Korea, South Korea, Japan	9
			An. lesteri Baisas and	(A)	China, Viet Nam	Guam, Japan,	4, 5, 6, 12
			Hu (= <i>anthropohagus</i>)			Philippines, South Korea	
			<i>An. paraliae</i> Sandosham	Л	Thailand, Viet Nam	Brunei, Malaysia,	4, 12
						Singapore	
		ı	An. peditaeniatus (Leicester	-) (E)	Cambodia, China,	Afghanistan, Bangladesh,	4, 6, 12
					Myanmar, Thailand,	India, Indonesia, Iran,	
					Viet Nam	Malaysia, Nepal, Pakistan,	
						Philippines, Sri Lanka	
Anopheles/	Nigerrimus		An. nigerrimus Giles s.l.	Л	Cambodia, China,	Bangladesh, Brunei,	4, 6, 12
Myzorhynchus/					Myanmar, Thailand,	India, Indonesia, Malaysia,	
Hyrcanus					Viet Nam	Nepal, Pakistan, Sri Lanka	
			<i>An. nitidus</i> Harrison,	D	Cambodia, China,	India, Indonesia, Malaysia	4, 12
			Scanlon and Reid		Myanmar, Thailand,		
					Viet Nam		
			<i>An. pursati</i> Laveran	С	Cambodia, Thailand,	Malaysia	4, 12
					Viet Nam		

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Table 2 (Continued).	

Subgenus/ Series/ Group	Subgroup	Complex	Speciesª	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)	Reference ^c
Anopheles/ Myzorhynchus/ Hyrcanus	Sinensis (new subgroup)	Sinensis	An. sinensis Wiedemann s.I.	A (A, E)	Cambodia, China, Myammar, Thailand, Viet Nam	India, Indonesia, Japan, Malaysia, Nepal, North Korea, Russia, Singapore, South Korea	4, 6, 12
		1	An. argyropus (Swellengrebel) s.I.	D	Cambodia, China, Lao PDR, Myanmar, Thailand, Viet Nam	India, Indonesia, Malaysia	4, 12
			<i>An. kweiyangensis</i> Yao and Wu	С	China		6, 12
			<i>An. kunmingensis</i> Dong and Wang	Л	China		6, 12
			An. liangshanensis Kang, Tan and Cao		China		6, 12
			<i>An. nimpe Nguyen</i> , Tran and Harbach		Viet Nam		12
			<i>An. pullu</i> s Yamada <i>An. vietnamensis</i> Nguyen, Tran and Nguven	F (A, F) U	China Vietnam	North and South Korea	6, 12 12
Cellia/ Neomyzom) Leucosphyrus	/ia/ Leucosphyrus	Leucosphyrus	An. introlatus Colless	D	Thailand	Indonesia, Malaysia	8, 9, 12
s -			<i>An. latens</i> Sallum and Pevton (<i>= leucophvrus</i> A)	(D, F)	Thailand	Indonesia, Malaysia	8, 9, 10, 12
		Dirus	<i>An. baimaii</i> Sallum and Peyton (= <i>dirus</i> D)	A	China, Myanmar, Thailand	Bangladesh, India	8, 9, 10, 12
			<i>An. cracen</i> s Sallum and Peyton (= <i>dirus</i> B)	D	Thailand	Indonesia, Malaysia	8, 9, 10, 12
			<i>An. dirus</i> Peyton and Harrison (= <i>dirus</i> A)	A (A)	Cambodia, China, Lao PDR, Thailand, Viet Nam		4, 8, 9, 10, 12
			An. nemophilous Peyton (= dirus F)		Thailand	Malaysia	4, 8, 9, 10, 12
			An. scanloni Sallum and Peyton (= <i>dirus</i> C)		Thailand		4, 8, 9, 10, 12

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Table 2 (Continued).

Subgenus/ Series/ Group	Subgroup	Complex	Speciesª	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)	Reference
Cellia/Neomyzomyia/	Hackeri		<i>An. hackeri</i> Edwards	Ъ	Thailand	Malaysia, Philippines	4, 9, 10, 12
reaccopilying			An. pujutensis Colless		Thailand	Indonesia, Malaysia	4, 9, 10, 12
Cellia/Neomyzomyia/	Riparis	ı	An. macarthuri Colless	С	Thailand	Malaysia	4, 9, 10, 12
Leucosphyrus Cellia/Neomyzomyia/ Kochi	,	ı	<i>An. kochi</i> Doenitz	C (A)	Cambodia, China, Lao PDR, Myammar,	Bangladesh, India, Indonesia, Malaysia,	4, 12
Cellia/Neomyzomyia/ Tessellatus			<i>An. tessellatus</i> Theobald	B (A, E, F)	Thailand, Viet Nam Cambodia, China, Lao PDR, Myanmar, Thailand, Viet Nam	Nepal, Philippines Bangladesh Guam, India, Indonesia, Malaysia, Maldives, Nepal, Philippines, Sri Lanka,	4, 12
						Taiwan	
Cellia/Myzomyia/ Funestus	Unassociated subgroup	Jeyporiensis	<i>An. jeyporiensis</i> James s.l.	(D, E)	Cambodia, China, Lao PDR, Myanmar, Thailand, Viet Nam	Bangladesh, India, Nepal, Taiwan	4, 12
Cellia/Myzomyia/	Minimus	Minimus	<i>An. minimus</i> Theobald	A (A)	Cambodia, China,	Bangladesh, India,	4, 12
Funestus			(= minimus A)		Lao PDR, Myanmar, Thailand, Viet Nam	Indonesia, Japan, Malaysia. Nepal, Pakistan, Sri Lanka, China (Taiwan)	
			<i>An. harrisoni</i> Harbach and Manguin (= <i>minimu</i> s C)		China, Myanmar, Viet Nam		12
		Fluviatilis	An. fluviatilis James s.l.		China, Myanmar, Viet Nam	Afghanistan, Bahrain, Bangladesh, India, Iran, Iraq, Kazakhstan, Nepal, Oman, Pakistan, Saudi Arahia Sri Janka	2, 11, 12
Cellia/Myzomyia/	Aconitus		<i>An. aconitus</i> Doenitz s.I.	А	Thailand	Indonesia	4, 12
		ı	<i>An. pampanai</i> Buttiker and Beales		Cambodia, Lao PDR, Thailand, Viet Nam		4, 12
		ı	An. varuna Iyengar	(F)	China, Lao PDR, Myanmar, Thailand, Viet Nam	Bangladesh, India, Indonesia, Nepal, Sri Lanka	4, 12

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			Table 2 (Co	ntinued).		
Subgenus/ Series/ Group	Subgroup	Complex	Species ^a	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)
Cellia/Myzomyia/ Funestus	Culicifacies	Culicifacies	An. culicifacies Giles	(Q)	Cambodia, China, Lao PDR, Myanmar (Burma), Thailand, Viet Nam	Afghanistan, Bangladesh, Ethiopia, Indi Nepal, Oman Sri Lanka. Ye
Cellia/Pyretophorus/ Ludlowae	ı	Sundaicus	<i>An. epiroticus</i> Linton and Harbach (<i>= sundaicus</i> A) <i>An. sundaicus</i> (Rodenwaldt) s.I.	B (A) B (A)	Cambodia, Thailand, Viet Nam China, Myanmar	Malaysia, Sir Bangladesh,
Cellia/Pyretophorus/ Subpictus		Subpictus	An. subpictus Grassi s.l.	(A, E, F)	Cambodia, China, Myanmar, Thailand, Viet Nam	Afghanistan, India, Indone Malaysia, Ma Islands, Nep Papua New C Philippines, S
		,	An. indefinitus (Ludlow)		Cambodia, China,	Indonesia, M

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Subgenus/ Series/ Group	Subgroup	Complex	Species ^a	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)	Reference ^c
Cellia/Myzomyia/ Funestus	Culicifacies	Culicifacies	An. culicifacies Giles	(Q)	Cambodia, China, Lao PDR, Myanmar (Burma), Thailand, Viet Nam	Afghanistan, Bahrain, 2 Bangladesh, Eritrea, Ethiopia, India, Iran, Iraq, Nepal, Oman, Pakistan, Sri Lanka, Yemen	9, 4, 12, 13
Cellia/Pyretophorus/ Ludlowae	·	Sundaicus	<i>An. epiroticus</i> Linton and Harbach (<i>= sundaicus</i> A) <i>An. sundaicus</i> (Rodenwaldt) s.I.	B (A) B (A)	Cambodia, Thailand, Viet Nam China, Myanmar	Malaysia, Singapore Bangladesh, India, Indonesia	1, 4
Cellia/Pyretophorus/ Subpictus		Subpictus	An subpictus Grassi s.l.	(A, E, F)	Cambodia, China, Myanmar, Thailand, Viet Nam	Afghanistan, Bangladesh, India, Indonesia, Iran, Malaysia, Maldives, Mariana Islands, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka	4, 12
			An. indefinitus (Ludlow) An. vagus Doenitz	U C (A, E)	Cambodia, China, Lao PDR, Thailand, Viet Nam Cambodia, China,	Indonesia, Malaysia, Mariana Islands, Nepal, Philippines Bangladesh, India,	4, 7, 12 4, 7, 12
Cellia/ Neocellia/ Annularis		,	<i>An. annulari</i> s Van der Wulp	C (A, E, F)	Cambodia, China, Myanmar, Thailand, Viet Nam	managan, marayara, Mariana Islands, Nepal, Philippines, Sri Lanka India, Indonesia, Malaysia, Philippines	4, 12
		- Nivines	An. philippinensis Ludlow An nivipes (Theobald) s I	В (A) С	Cambodia, China, Lao PDR, Myanmar, Thailand, Viet Nam China Lao PDR	Bangladesh, India, Indonesia, Lao PDR, Malaysia, Nepal, Philippines India Malavsia Nenal	4, 4, 12
Cellia/ Neocellia/ Jamesii			An jamesi Theobald s.l.		Thailand, Viet Nam Cambodia, China, Lao PDR, Myanmar, Thailand, Viet Nam	Bangladesh, India, Malaysia, Nepal, Sri Lanka	4, 12

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Table 2 (Continued).

Subgenus/ Series/ Group	Subgroup	Complex	Speciesª	Vector status in GMS (elsewhere) ^b	Distribution in GMS	Distribution elsewhere (Non-GMS)	Reference
	1	1	An. pseudojamesi Strickland and Choudhury	<u></u> : с	China, Myanmar, Thailand, Viet Nam	Bangladesh, India, Indonesia, Nepal	4, 12
			<i>An. splendidus</i> Koidzumi	D	China, Myanmar, Thailand, Viet Nam	Afghanistan, India, Nepal, Pakistan	4, 12
Cellia/ Neocellia/ Maculatus	Maculatus	ı	An. dravidicus Christophers (= maculatus C)	Ъ	China, Lao PDR, Myanmar, Thailand	India, Nepal	4, 12, 13
		Maculatus	<i>An. maculatus</i> Theobald (= <i>maculatus</i> B)	B (A, F)	Cambodia, China, Lao PDR. Mvanmar,	Bangladesh, India, Indonesia. Malavsia.	4, 12, 13
					Thailand, Viet Nam	Nepal, Pakistan, Philippines	
			An. maculatus Theobald s.l.	С	Thailand	Malaysia	4, 12
Cellia/ Neocellia/	Sawadwongporni		An. notanandai	Л	Lao PDR, Thailand		4, 12
Maculatus			Rattanarithikul and Green (= <i>maculatus</i> G)				
		ı	An. sawadwongporni	с	China, Lao PDR,		4, 12, 13
			Rattanarithikul and Green		Myanmar, Thailand,		
			(= maculatus A)				
	Unassociated subgroup		<i>An. pseudowillmori</i> (Theobald) (= <i>maculatus</i> I)	A	China, Lao PDR, Thailand, Viet Nam	India, Nepal	4, 12
			An. willmori (James)	(A)	China, Myanmar,	Bangladesh, India,	4, 12
			(= maculatus H)		Thailand	Nepal, Pakistan	
Cellia/ Neocellia/	ı	Karwari	<i>An. karwari</i> James s.l.	B (D)	Cambodia, China,	Bangladesh, India,	4, 12
Unassociated group					Lao PDR, Myanmar,	Indonesia, Malaysia,	
					Thailand, Viet Nam	Nepal, Philippines, Sri Lanka	
		ı	<i>An. stephensi</i> Liston	(A)	China, Myanmar, Thailand	Afghanistan, Bahrain, Bangladesh, Egypt, India, Iran. Iraq. Neoal. Oman.	4, 12
						Pakistan, Saudi Arabia	

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7 (Rueda et al, 2011), 8 (Sallum et al, 2005a), 9 (Sallum et al, 2005b), 10 (Sallum et al, 2007), 11 (Subbarao et al, 1994), 12 (WRBU, 2012), 13 (WHO, 2007). °References: 1 (Linton et al, 2005), 2 (Harbach, 2004), 3 (Manguin, 2008b), 4 (Rattanarithikul et al, 2006), 5 (Rueda et al, 2005a, b), 6 (Rueda et al, 2005), 5 (Rueda et al, 2005), 5 (Rueda et al, 2005), 6 (Rueda et al, 2005), 6 (Rueda et al, 2005), 7 (Rueda et al, 2005)

D = noted as vector, but unspecified sprozoites or oocysts); Japanese encephalites, E; Filariasis, F; non-vector, U.

^aSensu lato (s.I.) means 'in the broad sense', *ie*, any or all members of the species complex. ^bVector status for malaria, A-D (A = sporozoites in the salivary glands, B = oocysts, C = ELISA,

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Species	Cambodia	China	Lao PDR	Mvanmar	Thailand	Viet Nam	Type locality ^c
An. aconfus x ^a x x A, B, C (r) x Kajoe Tanam, Sumata, Indonesia An. anudris x x x A, B, C (r) x Nount Ardjeeno, Java, Indonesia An. anudris x x x x x x Nount Ardjeeno, Java, Indonesia An. anudris x x x x Nount Ardjeeno, Java, Indonesia An. anudris x x x Nount Ardjeeno, Java, Indonesia An. anudris x x Nount Ardjeeno, Java, Indonesia An. cauloffactes x Nount Ardjeeno, Java, Indonesia An. faus* x x Nount Ardjeeno, Java, Indonesia An. faus* x Nount Ardjeeno, Java, Indonesia An. faus* x X Nount Ardjeeno, Java, Indonesia An. faus* x X Nount Ardjeeno, Java, Indonesia An. faus*								
An. argyropus X X X, B, C (r) X Dell, Sumatra, Indonesia An. arrutaris X X X X X Dell, Sumatra, Indonesia An. arrutaris X X X X X Mount Ardjoeno, Java, Indonesia An. arrutaris X X X A, B, C (r) X Mount Ardjoeno, Java, Indonesia An. arrutaris X X A, B, C (r) X Mount Ardjoeno, Java, Indonesia An. arrutaris X X A, B, C (r) X Mount Ardjoeno, Java, Indonesia An. arrutaris X X A, B, C (r) X A, B, C (r) Mount Ardjoeno, Java, Indonesia An. arrutaris X X X A, B, C (r) X A, B, C (r) Mount Ardjoeno, Java, Indonesia An. arrutaris X X X A, B, C (r) X A, B, C (r) Mount Ardjoeno, Java, Indonesia An. arrutaris X X A, B, C (r) X A, B, C (r) Mount Ardjoeno, Java, Indonesia An. arrutaris X X A, B, C (r) X B, C (r)	An. aconitus	X ^a	×	×	×	A, B, C ^b	×	Kajoe Tanam, Sumatra, Indonesia
An. anrularis x x x x x mount Ardjoeno, Java, Indonesia An. barbriostris x x x x Mount Ardjoeno, Java, Indonesia An. barbriostris x x x x Mount Ardjoeno, Java, Indonesia An. culticifacies x x x A, B (T) x Mount Ardjoeno, Java, Indonesia An. culticifacies x x A, B (T) x Mount Ardjoeno, Java, Indonesia An. culticifacies x D(w) x A, B (T) x Mount Ardjoeno, Java, Indonesia An. dirus* D(w) x A, B (T) X Mount Ardjoeno, Java, Indonesia An. dirus* D(w) x A, B (T) X Java Malaysia An. dirus* A B (T) x Java Mount Ardjoeno, Java, Indonesia An. dirus* X X A, B (T) X Java Mount Ardjoeno, Java, Indonesia An. diversi X A, B (T) X A, B (T) X Java Mount Ardjoeno, Java, Indonesia An. diversi X <	An. argyropus	×	×	P	×	A, B, C (r)	×	Deli, Sumatra, Indonesia
An. barbinostris x x A. B. C (r) x Mount Ardjoeno, Java, Indonesia An. carwfordi x x x x x kull Lumpur, Malaysia An. carwfordi x x x x x kull Lumpur, Malaysia An. carwfordi x x x x x kull Lumpur, Malaysia An. carwfordi x x x x x kull Lumpur, Malaysia An. carwfordi x x x x x kull Rumpur, Malaysia An. jerzenin Lumpur, x x x x x x kull Rumpur, Malaysia An. jerzenin Lumpur, malaysia x x x x cullon. Travancore. India An. jerzenin Lumpur, malaysia x x x x cullon. Travancore. India An. ancuratus* B (r) x A. B. (r) x cullon. Travancore. India An. necorabytus* - - A. B. (r) x cullon. Travancore. India	An. annularis	×	×	×	×	×	×	Mount Ardjoeno, Java, Indonesia
An. crawfordi x x x A, B (r) x Kuala Lumpur, Malaysia An. cultificates x x x x x, B, C, D, F (w) How and Burb Pharam, Trachin Buri, Thaland An. ciltoria x x x x x x x x and nuck An. jeryportensis x x x x x x and nuck	An. barbirostris	×	×	×	×	A, B, C (r)	×	Mount Ardjoeno, Java, Indonesia
An. culrifractesxxxA. B. (r)B (m)Hoshangabad, IndiaAn. culrifractesxxxA, B, C, D, F (w)A, B, C, D, T (w)B m Bu Pharam, Prachin Bur AndinaAn. dirus*D (w)xxxA, B, C, D (r)xQ (w)B m Bu Pharam, Prachin Bur AndinaAn. dirus*xxxxxA, B, C, D (r)xQ (w)B m Bu Pharam, Prachin Bur AndinaAn. dirus*xxxxA, B, C, D (r)xJwpur State (Maharashtra state)An. fervaribA, B, C, D (r)xJwpur State (Maharashtra state)An. fervariB (w)xA (w)B (w)A (w)An. naculatus*B (w)xA (w)B (w)An. maculatus*B (w)xA (w)An. maculatus*B (w)xA, B, C, D (w)A, B, L (w)Hong Kong, China-An. minusA (w)xA, B (r)A, B (r)C alcuta, IndiaAn. minusA (w)xA, B (r)A, B (r)An. minusA (w)xA (w)A, B (r)An. minusA (w)xA (w)A, B (r)An. minusA (w)xA (w)A (w)A (w)-<	An. crawfordi	×	×			A, B (r)	×	Kuala Lumpur, Malaysia
An. dirus ⁶ D (w) x x D (w) x x D (w) x x D (w) x x Quilon, Travancore, India An. jamesiji x x x x x x Quilon, Travancore, India An. jamesiji x x x x x Quilon, Travancore, India An. jeyporiensis x x x x x Quilon, Travancore, India An. <i>Ieucosphyrus¹</i> - - - - A, B, C, D (r) x Quilon, Travancore, India An. <i>Ieucosphyrus¹</i> - - - - A(r) - Kajoe Tanam, Sumatra, Indonesia An. <i>minus</i> B (w) x A, C (w) A, B, C (r) A, B, I (w) Hong Kong, China An. <i>mingeritus</i> - - - - A, B (r) - Kajoe Tanam, Sumatra, Indonesia An. <i>mingeritus</i> - - A, B (r) - Kajoe Tanam, Sumatra, Indonesia An. <i>mingerinus</i> -	An. culicifacies	×	×		×	A, B (r)	B (w)	Hoshangabad, India
An. jamesixxxA, B, (r)xQuilon, Travancore, IndiaAn. jeyporiensisxxxxA, B, C, D (r)xJeypur State (Manarashtra state)An. jeyporiensisxxxA, B, C, D (r)xJeypur State (Manarashtra state)An. jeyporiensisxxxA, B, C (r)xA, B, C (r)An. leucosphyrusA (r)-Kawar, Bomby, IndiaAn. neculatusB (w)xX, B, C (w)A, B, C (r)A, B, I (w)Hong Kong, ChinaAn. maculatusB (w)xA, C (w)A, B, C (w)A, B, C (w)A, B, C (w)A, B, C (w)An. minimusA (w)xA, C (w)A, B, C (w)A, B, C (w)A, B (r)-Calcuta, IndiaAn. minimusA (w)xXXXXXSan Jose, Abra, Luzon, PhilippinesAn. niperimusxxxXXXXSan Jose, Abra, Luzon, ChinaAn. niperimusxXXXXXSan Jose, Abra, Luzon, ChinaAn. subpictusxXXXXSan Jose, Abra, Luzon, ChinaAn. subpictusxXXX <td>An. dirus^e</td> <td>D (w)</td> <td>×</td> <td>×</td> <td>D (w)</td> <td>A, B, C, D, F (w)</td> <td>A (w)</td> <td>Ban Bu Pharam, Prachin Buri, Thailand</td>	An. dirus ^e	D (w)	×	×	D (w)	A, B, C, D, F (w)	A (w)	Ban Bu Pharam, Prachin Buri, Thailand
An jeyporiensisxxxA, B, C, D (r)xJeypur State (Maharashtra state) and Nagpur Orissa state), IndiaAn. <i>karwari</i> A, B, C (r)-Karwar. Bombay. IndiaAn. <i>karwari</i> Karwar. Bombay. IndiaAn. <i>karwari</i> Karwar. Bombay. IndiaAn. <i>macuosphrus'</i> Karwar. Bombay. IndiaAn. <i>minuus</i> B (w)×A, B, C (w)A, B, C (w)A, B, C (w)Hong Kong. ChinaAn. <i>minuus</i> A (w)×A, C (w)A, B (r)A, C (w)Hong Kong. ChinaAn. <i>minuus</i> A, B (r)-Calcuta, IndiaAn. <i>minus</i> A, B (r)-Calcuta, IndiaAn. <i>minus</i> An. <i>minus</i> An. <i>minus</i> An. <i>minus</i> An. <i>minus</i> </td <td>An. jamesii</td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td>A, B (r)</td> <td>×</td> <td>Quilon, Travancore, India</td>	An. jamesii	×	×	×	×	A, B (r)	×	Quilon, Travancore, India
An. KarwariA. B, C (r)-Karwar Kanbay, IndiaAn. KarwariKarwar, Bombay, IndiaAn. mieuusB (w)××A(r)-Karwar, Bombay, IndiaAn. mieuusB (w)×A, C (w)A, B, C, B, H, K (r)A, B, 1 (w)Hong Kong, ChinaAn. mieuusIAn. mieuusAn. nivpesA, B (r)×Kuala Lumpur, MalaysiaAn. nivpesAn. subicus	An. jeyporiensis	×	×	×	×	A, B, C, D (r)	×	Jeypur State (Maharashtra state)
An. karwariA, B, C (r)-Karwar, Bombay, IndiaAn. kacosphytustA (r)-Kajoe Tanam, Sumatra, IndonesiaAn. kacosphytustA (r)-Kajoe Tanam, Sumatra, IndonesiaAn. maculatustB (w)×A (r)-Kajoe Tanam, Sumatra, IndonesiaAn. maculatustB (w)×A, C (w)A, B, C, D (w)A, B, I (w)Hong Kong, ChinaAn. migerimusA, B (r)-C (w)An. nigerimusA, B (r)-C (w)An. nivipesA, B (r)-C (w)An. sinensis××A, B (r)×San Jose, Abra, Luzon, PhilippinesAn. subjictus××A, B (r)×A (w)IndiaAn. subjictus×A (w)×A (w)M (w)KanagranAn. subjictus××A (w)×A (w)IndiaAn. subjictus×A (w)×A (w)IndiaAn. subjictus×A (w)×A (w)IndiaAn. subjictus××A (w)×A (w)An. subjictus×A (w)×A (w)IndiaAn. subjictus×A (w)×A (w)IndiaAn. subjictus××A (w)×A (w)An. surdacush×A (w)×A (w) <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>and Nagpur (Orissa state), India</td>								and Nagpur (Orissa state), India
An. leucosphyrus' - - - A(r) - Kajoe Tanam, Sumatra, Indonesia An. maculatus ⁹ B (w) × × A, B, C, (w) A, B, C, (B, H, I, K) Hong Kong, China An. maculatus ⁹ B (w) × A, C (w) A, B, C, (w) A, B, C, (w) A, B, C, (w) Hong Kong, China An. minimus A (w) × A, C (w) A, B, C, (w) A, B, C, (w) Hong Kong, China An. migerimus - - - - A, B (r) × Kuala Lumpur, Malaysia An. nivipes - - - A, B (r) × Kuala Lumpur, Malaysia An. nivipes - - - A, B (r) × San Jose, Abra, Luzon, Philippines An. nivipes - - - A, B (r) × San Jose, Abra, Luzon, Philippines An. nivipes - - - - A, B (r) × San Jose, Abra, Luzon, Philippines An. nivipes - - - - - - - - An. subpicturs × - -<	An. karwari					A, B, C (r)		Karwar, Bombay, India
An. maculatus ⁴ B (w)xxA, B, C, WA, B, I, WHong Kong, ChinaAn. minimusA(w)xA, C (w)A, B, C, D (w)A, C (w)Hong Kong, ChinaAn. migerimusA, B (r)-Calcutta, IndiaAn. nigerimusA, B (r)-Calcutta, IndiaAn. nigerimusA, B (r)-Calcutta, IndiaAn. nigerimusCalcutta, IndiaAn. nivpessCalcutta, IndiaAn. nivpessCalcutta, IndiaAn. nivpessCalcutta, IndiaAn. nivpessCalcutta, IndiaAn. nivpessCalcutta, IndiaAn. nivpessx <t< td=""><td>An. leucosphyrus^f</td><td>,</td><td></td><td>,</td><td></td><td>A (r)</td><td></td><td>Kajoe Tanam, Sumatra, Indonesia</td></t<>	An. leucosphyrus ^f	,		,		A (r)		Kajoe Tanam, Sumatra, Indonesia
An. minimusA(w)xA, C(w)C, D(w)A, C(w)Hong Kong, ChinaAn. nigerrimusA, B (r)-Calcutta, IndiaAn. nivipesA, B (r)xKuala Lumpur, MalaysiaAn. nivipesA, B (r)xCalcutta, IndiaAn. nivipesA, B (r)xCalcutta, IndiaAn. nivipesxxxxxSan Jose, Abra, Luzon, PhilippinesAn. nivipesxxxA, B (r)xGuangzhou (=Canton), ChinaAn. subpictusxxB, C, D (r)xIndiaAn. subpictusxANNNNAn. subpictusxA(w)xA(w)IndiaAn. subpictusxANNNNAn. subpictusxA(w)XA(w)IndiaAn. vagusxANNNNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn. vagusxA(w)XA(w)NNAn.	An. maculatus ^g	B (w)	×	×	A, B, C (w)	A, B, C, E, G, H, I, K (r)	A, B, I (w)	Hong Kong, China
An. nigerrimusA, B (r)-Calcutta, IndiaAn. nivipes-××××Kuala Lumpur, MalaysiaAn. nivipes-××××Kuala Lumpur, MalaysiaAn. philippinensis×××××San Jose, Abra, Luzon, PhilippinesAn. subpictus××××A, B (r)×Guangzhou (=Canton), ChinaAn. subpictus×××A, W)×IndiaAn. subpictus××A, W)×A, W)IndonesiaAn. vagus××A, W)×A, W)×An. vagus××A, W)×Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia**A, W×A, W)×Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia***A, W×A, W)×Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia****A, W×A, W)* </td <td>An. minimus</td> <td>A (w)</td> <td>×</td> <td>A, C (w)</td> <td>C (w)</td> <td>A, B, C, D (w)</td> <td>A, C (w)</td> <td>Hong Kong, China</td>	An. minimus	A (w)	×	A, C (w)	C (w)	A, B, C, D (w)	A, C (w)	Hong Kong, China
An. nivipes-××-A, B (r)×Kuala Lumpur, MalaysiaAn. philippinensis××××××San Jose, Abra, Luzon, PhilippinesAn. sinensis××××A, B (r)×Guangzhou (=Canton), ChinaAn. subpictus×××B, C, D (r)×IndiaAn. subpictus××A(w)×A(w)IndiaAn. subpictus××A(w)×A(w)IndiaAn. subpictus××A(w)×A(w)IndiaAn. subpictus××A(w)×A(w)IndiaAn. subpictus××A(w)×A(w)IndiaAn. subgictus××A(w)×A(w)IndiaAn. subgictus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus××A(w)×A(w)IndiaAn. vagus×××A(w)×Y(w)Y(w)An. vagus×××A(w)×A(w)Y(w)An reacent, but with nukn	An. nigerrimus					A, B (r)		Calcutta, India
An. philippinensisxxxxSan Jose, Abra, Luzon, PhilippinesAn. sinensisxxxA, B (r)xGuangzhou (=Canton), ChinaAn. subpictusxxxB, C, D (r)xIndiaAn. subpictusxxA(w)A, W)IndiaAn. subpictusxxA(w)XA(w)An. subpictusAxA(w)A(w)A(w)An. subaicushAxA(w)XBarjoe-Biroe, Java, IndonesiaAn. vagusxxA, B (w)XA(w)A(w)An. vagusxXA(w)A(w)A(w)A(w)An. vagusxA, XXA(w)A(w)A(w)An. vagusxA, XXA(w)A(w)A(w)An. vagusxA, XXA(w)A(w)A(w)An. vagusxA, XXA, B(w)A(w)A(w)An. vagusxA, B(w)XA(w)A(w)A(w)An. vagusxA, B(w)XA(w)A(w)A(w)An vagusxA, B(w)XA(w)A(w)A(w)An vagusxA, B(w)XA(w)A(w)A(w)An vagusxXA, B(w)XA(w)A(w)An vagusxXXA, B(w)A(w)A(w)An vagusxXXA, B(w)A(w)A(w)An c	An. nivipes		×	×		A, B (r)	×	Kuala Lumpur, Malaysia
An. sinensisxxA, B (r)xGuangzhou (=Canton), ChinaAn. subpictusxxxB, C, D (r)xIndiaAn. subpictusxxxB, C, D (r)xIndiaAn. sundaicus ^h AxA(w)A(w)A(w)IndonesiaAn. sundaicus ^h AxxA(w)xBanjoe-Biroe, Java, IndonesiaAn. vagusxxA, B (w)xA(w)A(w)An. vagusxxA, B (w)xA(w)IndonesiaAn. vagusxxxA, B (w)xA(w)An. vagusxxA, B (w)xA(w)IndonesiaAn. vagusxxA, B (w)xA(w)IndonesiaAn. sudicusxxA, B (w)xA(w)A(w)An. vagusxxA, B (w)xA(w)Indonesia**A, B (w)xA, B (w)xA(w)**A, B (w)xA, B (w)xA(w)***A, B (w)xA(w)Indonesia***Banjoe-Biroe, Java, Indonesia*******A, B (w)*************************** </td <td>An. philippinensis</td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td>San Jose, Abra, Luzon, Philippines</td>	An. philippinensis	×	×	×	×	×	×	San Jose, Abra, Luzon, Philippines
An. subpictusxxxB, C, D (r)xIndiaAn. sundaicushAxxA(w)A(w)NNonesiaAn. vagusxxxA(w)xA(w)IndonesiaAn. vagusxxxA(w)xA(w)IndonesiaAn. vagusxxxA(w)xA(w)NonesiaAn. vagusxxxA(w)xA(w)IndonesiaAn. vagusxxxA(w)xA(w)Indonesia**xxxA(w)xA(w)Indonesia**xxxA(w)xXIndonesia***A(w)xxA(w)XX***XXA(w)xX***XXXYY****A(w)XYY*****XYY******YY*******Y*************************************<	An. sinensis	×	×		×	A, B (r)	×	Guangzhou (=Canton), China
An. sundaicus ^h A x A(w) N N Indonesia An. vagus x x x A, B (w) x Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia An. vagus x x x A, B (w) x Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia * * * Periode Kock, Sumatra and Banjoe-Biroe, Java, Indonesia * * * * * * * * * * * * * * * * * * * * * * <t< td=""><td>An. subpictus</td><td>×</td><td>×</td><td>×</td><td>×</td><td>B, C, D (r)</td><td>×</td><td>India</td></t<>	An. subpictus	×	×	×	×	B, C, D (r)	×	India
An. vagus x x x Fort de Kock, Sumatra and Banjoe-Biroe, Java, Indonesia * * Present, but with unknown sibling species composition. ^b Reference for species complex: r (Rattanarithikul <i>et al.</i> , 2006), w (WHO, 2007). ^c Type locality is the original place where holotype or type specimen was found that served as basis to describe new species. The genetic or chromosomal forms (<i>eg</i> , A-K) must be compared with specimens from the type locality to determine if they are conspecific or undescribed species. ^d No record. ^e An. <i>dirus</i> complex: An. <i>dirus</i> s.s. (<i>= dirus</i> sp A), An. cracens (<i>= dirus</i> sp B), An. scanloni (<i>= dirus</i> sp C) and An baimaii (<i>= dirus</i> sp D), An. nemaphilous (<i>= dirus</i> sp F). ^t An. leucosphyrus complex: An. <i>leu</i> .	An. sundaicus ^h	۷	×	A (w)	×	A (w)	A (w)	Indonesia
^a x = present, but with unknown sibling species composition. ^b Reference for species complex: r (Rattanarithikul <i>et al</i> , 2006), w (WHO, 2007). ^c Type locality is the original place where holotype or type specimen was found that served as basis to describe new species. The genetic or chromosomal forms (<i>eg</i> , A-K) must be compared with specimens from the type locality to determine if they are conspecific or undescribed species. ^d No record. ^e An. <i>dirus</i> complex: An. <i>dirus</i> s.s. (<i>= dirus</i> sp A), An. <i>cracens</i> (<i>= dirus</i> sp B), An. scanloni (<i>= dirus</i> sp C) and An baimaii (<i>= dirus</i> sp D), An. nemaphilous (<i>= dirus</i> sp P), ^t An. leucosphyrus complex: An. <i>leu</i> -	An. vagus	×	×	×	×	A, B (w)	×	Fort de Kock, Sumatra and
^a x = present, but with unknown sibling species composition. ^b Reference for species complex: r (Rattanarithikul <i>et al</i> , 2006), w (WHO, 2007). ^c Type locality is the original place where holotype or type specimen was found that served as basis to describe new species. The genetic or chromosomal forms (<i>eg</i> , A-K) must be compared with specimens from the type locality to determine if they are conspecific or undescribed species. ^a No record. ^e An. <i>dirus</i> complex, An. <i>dirus</i> s.s. (<i>= dirus</i> sp A), An. <i>cracens</i> (<i>= dirus</i> sp B), An. scanloni (<i>= dirus</i> sp C) and An baimaii (<i>= dirus</i> sp D), An. nemaphilous (<i>= dirus</i> sp F). ^t An. leucosphyrus complex: An. <i>leu</i> -								Banjoe-Biroe, Java, Indonesia
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Table 3

epiroticus (= sundaicus A).

(= maculatus C), An. notonandai (= maculatus G), An. willmori (= maculatus H), An. pseudowillmori (= maculatus I). ^hAn. sundaicus complex: sundaicus s.s., An.



study by Sallum *et al* (2005a). For example, the cibarial armature is indistinguishable among the four species examined and was therefore not useful for separating the species from each other (Somboon *et al*, 2009). The authors also noted that the cibarial armature of the other species in the Dirus complex, *An. nemophilous*, *An. elegans* and *An. takasagoensis* was not known, but this feature could be used to distinguish from at least one member of the Leucosphyrus complex, *An. leucosphyrus* as reported by Anthony *et al* (1999). Generally, members of the Leucosphyrus complex can be distinguished easily from those of the Dirus complex in having the accessory sector pale wing spot present on veins C, subcosta and R, and by the absence of pale scales at the base of hind tarsomere 4 (Rattanarithikul *et al*, 2006).

Sallum *et al* (2005b) reviewed several studies using genetic and molecular tools to investigate species recognition, gene flow, and genetic population structure of members of the Leucosphyrus Group. Two allele-specific-polymerase chain reaction (PCR) assays were developed to distinguish and unambiguously identify *An. dirus, An. cracens, An. scanloni, An. baimaii* and *An. nemophilous* using an ITS2 sequence (Walton *et al*, 1999) and a SCAR-based PCR (SCAR: Sequence characterized amplified region) (Manguin *et al*, 2002). In Cambodia, Ma *et al* (2005) used rDNA ITS2 and D3 sequences to identify molecular variants of *An. dirus* s.s. (*= An. dirus* A) and *An. minimus* s.s. (*= An. minimus* A).

Using mitochondrial COI and ND6 genes to study the phylogenetic relationships among 13 of the 20 species of the Leucosphyrus Group of *Anopheles (Cellia)*, Sallum *et al* (2007) revealed that the current classification of Leucoscphyrus Subgroup was composed of unnatural assemblages. The results also revealed the monophyly of the Leucosphyrus Group and the Hackeri and Riparis Subgroups (The Riparis subgroup consists of *An. riparis* King and Baisas, *An. cristatus* King and Baisas, *An. marcarthuri* Colless); however the Leucosphyrus Subgroup and the Leucosphyrus complex were regarded as polyphyletic.

Recent in-depth phylogeographic analysis on 269 individuals from 21 populations of the two widespread species in the Dirus complex (*An. dirus* and *An. baimaii*) from mainland Southeast Asia revealed that the population history of *An. baimaii* is far more complex than previously thought (O'Loughlin *et al*, 2008). It was concluded that "the long and complex population of these anthropophilic species suggests their expansions are not in response to the relatively recent [ca 40 kyr BP (kilo years before present)] human expansions in mainland Southeast Asia but, rather fit well with our understanding of Pleistocene climatic change there" (O'Loughlin *et al*, 2008).

1.1.1 Mixed infections, simian malaria vectors and sporozoite rates. Compared to most species in the Leucosphyrus Group that have infrequent contact with humans, a few members are regarded as efficient malaria vectors in southern and Southeast Asia (Sallum *et al*, 2005a) including the GMS. In a review to assess the occurrence of mixed

malaria species infections among 11,289 *Anopheles* in Thailand and 596 anopheline specimens of *An. dirus, An. minimus* and *An. maculatus* from the North West border of Thailand, mixed *P. falciparum* (Pf) and *P. vivax* (Pv) infections were not found (Imwong *et al*, 2011). There were 23 Pf (0.20%) and 24 Pv (0.21%) infections, and both VK210 and 247 *P. vivax* genotypes were distinguished by Malaria Antigen Panel (V-MAP[®]) assay which is a rapid, one-step procedure for the detection of malaria sporozoite antigens in mosquitoes (Sattabongkot *et al*, 2004b). Only one *P. malariae* infective specimen was from mainland Southeast Asia. The lack of mixed infections may be explained by very low transmission intensities in most of Southeast Asia (Entomological Inoculation Rate or EIRs typically <1/year) which reduce the probabilities of separate inoculations within a narrow time window (Gingrich *et al*, 1990). In Lao PDR, ELISA sporozoite rates of *An. dirus* were 1.45% and 2.56% in August and October 2002, respectively, including a high number of oocysts ranging from 1 to 250 were found in a dissected midgut (Vythilingam *et al*, 2005b).

A recent evaluation showed that V-MAP[®] assay provided performance close to that achieved with the circumsporozoite enzyme-linked immunosorbent assay (CSP-ELISA) (Sattabongkot et al, 2004a). Using laboratory-reared An. dirus specimens and a cut-off of 150 P. falciparum sporozoites, sensitivity, specificity and accuracy values were 100%, 99.2% and 0.99, respectively. For P. vivax variant 210, optimal performance was also achieved using a cut-off of 150 sporozoites (sensitivity=94.8%, specificity = 94.5% and accuracy= 0.95). Using a cut-off of 30 pg P. vivax variant 247 (mosquitoes with less than this amount of antigen were considered negative), the assay performance (sensitivity=94.3%, specificity = 99.2% and accuracy= 0.99) was comparable to that achieved for P. falciparum and P. vivax 210 (Sattabongkot et al, 2004b). The assay was sensitive and specific for each of the parasites evaluated, simple to use, rapid (results obtained in 15 minutes) and could be used by field workers for conducting rapid surveys of malaria vectors or in humanitarian or military operations to assess the medical threat. However, Durnez et al (2011) showed that high rates of false positives can occur with the CSP-ELISA method, especially when tested for P. falciparum on vectors having a zoophilic biting trend present in Cambodia and Viet Nam. Overestimation of EIR in dominant and secondary vectors may be avoided by confirming all positive CSP-ELISA results by a second CSP-ELISA test on the heated ELISA lysate or by performing *Plasmodium* specific PCR followed, if possible by sequencing of the amplicons for *Plasmodium* species determination (Durnez et al, 2011).

In Southeast Asia, Imwong *et al* (2011) estimated that approximately one-third of patients treated for Pf malaria experienced a subsequent Pv infection with a time interval suggesting relapse. Due to the inadequate representation of vectors transmitting malaria (Imwong *et al*, 2011) and the low EIRs (Gringrich *et al*, 1990; Snounou and White, 2004), the majority of these mixed species malaria infections are acquired from separate inoculations. The entomological evidence suggests that mixed species malaria infections acquired by simultaneous inoculations of sporozoites from multiply infected anopheline mosquitoes are relatively rare (Imwong *et al*, 2011). [Simultaneous inoculation is defined as occurring either through the same effective bite or through two consecutive bites in quick succession, within one week of each other (Snounou and White, 2004)].

In Viet Nam, sporozoite-positive bites of *An. dirus* were reported before 09:00 PM (Van Bortel *et al*, 2010) and co-infections of *P. knowlesi*, *P. falciparum*, *P. vivax* and *P. malariae* (Nakazawa *et al*, 2009; Marchand *et al*, 2011) in *An. dirus* were reported for the first time. It is suspected that the commonly found macaques in the forests of Khanh Phu are bitten by the same *An. dirus* population that bites humans. Further studies are required to determine if these monkeys are zoonotic malaria reservoirs of *P. knowlesi* or if the parasite is also transmitted from person to person, and to clarify if *P. knowlesi* is the only malaria parasite in monkeys transmitted by *An. dirus* (Marchand *et al*, 2011). Although cases of *P. knowlesi* have been reported from Thailand, the vector remains unknown and the role of *An. cracens* is yet to be established (Saeung, 2012).

1.1.2 Vector-parasite host interaction and transmission-blocking studies. Membrane feeding apparatus was used for seven separate studies to evaluate: a) the infectiousness of *P. falciparum* and *P. vivax* carriers to *An. dirus* mosquitoes (Coleman *et al*, 2004); b) the blocking of transmission to *An. dirus* mosquitoes by antibody to *P. vivax* malaria vaccine candidates Pvs25 and Pvs28 (Sattabongkot *et al*, 2003b); c) several methods of preparing gametocytemic blood collected from patients naturally infected with *P. vivax* for membrane feeding and compared with direct feeding (Sattabongkot *et al*, 2003a); d) sporontocidal activity of tafenoquine (WR-238605) and artelinic acid against naturally circulating isolates of *P. vivax* (Ponsa *et al*, 2003); e) the existence of short- and long-term antibody responses (IgMs and IgGs) against anopheline mosquito salivary gland proteins in people from malaria endemic regions (Waitayakul *et al*, 2006); f) the efficacy of using out-of-date preserved human blood for colonizing *An. dirus* and *Ae. aegypti* (Linnaeus) (Pothikasikorn *et al*, 2010); and g) the susceptibility of *Anopheles* and culicine mosquitoes to nocturnal subperiodic *Wuchereria bancrofti* (Cobbold) (Pothikasikorn *et al*, 2008).

Two of these experiments are epidemiologically relevant to malaria transmission in western Thailand. In experiment (c), Sattabongkot *et al* (2003a) found that sera from symptomatic adult patients significantly reduced mosquito infection rates as well as oocyst rates and density, suggesting a possible transmission-blocking antibody (Coleman *et al*, 2004). As seen in experiment (a), high levels of transmission-blocking antibody from sera of asymptomatic children and adults may account for the relative low mosquito infection rates, *eg*

4.5% and 2.9% oocyst rates from infectious Pf and Pv samples (Coleman *et al*, 2004). In western Thailand, the infectious reservoirs comprise the probabilities of 1 in 6,700 and 1 in 5,700 mosquitoes becoming infected with Pf and Pv, respectively after feeding on a single human. Although factors most commonly found to be associated with vector abundance were relatively stable across the seven hamlets in the Thai-Myanmar border, changes in the community could potentially affect transmission probabilities for individuals in the population (Lawpoolsri *et al*, 2010). Experimental membrane feedings (study f, see above) using *An*. *dirus* colony show that there is no disadvantage in using outdated preserved human blood samples over fresh blood as a nutrition source for rearing mosquitoes in the laboratory (Pothikasikorn *et al*, 2010).

Pothikasikorn *et al* (2008) showed for the first time that three *Anopheles* species [from established colonies of *An. dirus* s.s. (*=dirus* A), *An. maculatus* and *An. minimus*] and one each of *Culex, Aedes,* and *Mansonia* species were capable of developing nocturnal subperiodic *Wuchereria bancrofti.* The two *Anopheles* species, *An. maculatus* and *An. minimus*, demonstrated significantly greater susceptibility to *W. bancrofti* than the two *Aedes* species regarded as natural vectors (Pothikasikorn *et al*, 2008). As human malaria and lymphatic filariasis (and possibly monkey malaria) are transmitted by the same vectors in Thailand and the rest of GMS, both diseases can be jointly controlled since they share a large proportion of their target population, and the national elimination programs have similar goals and strategies. National policies which include the Integrated Vector Management (IVM) concept allows programs to control malaria and lymphatic filariasis through effective coordination and so benefit from each other program's activities, thus enhancing their overall impact on public health. IVM is a system of rational decision-making developed to optimize the use of resources for vector control (WHO 2004, 2008).

1.1.3 Immature stages. Species of the Dirus complex inhabit forested mountains and foothills, cultivated forests, rubber plantations and forest fringes. Typical larval habitats include small, shallow, usually temporary, mostly shaded bodies of fresh, stagnant (or very slowly flowing) water, such as pools, puddles, small gem pits, animal footprints (*eg* elephant or buffalo footprints), wheel ruts, hollow logs, streams, and wells located in primary and secondary evergreen or deciduous forests, bamboo forests and fruit or rubber plantations. Water habitats can be clear or turbid with nitrogenous waste, due to animal excreta or rotten leaves. These species are most abundant during the rainy (monsoon) season (Rattanaritikhul *et al*, 2006; Sinka *et al*, 2011).

Soil analysis showed that flowing drainage, wet season, high alkalinity, aluminum and magnesium were associated with the presence of *An. dirus, An. maculatus* and *An. minimus* in nature (Kankaew *et al,* 2005). In Mae Sot District of Tak Province in Thailand,

Kengluecha *et al* (2005a, b) showed a significantly negative relationship between pH and *An. dirus* density. *Anopheles dirus* larvae were found in habitats with lower pH values, especially in ground pools. Using GIS and aerial photographs in seven provinces in Thailand, Lek-Uthai *et al* (2010) showed that the proportions of aquatic arthropods were significantly different from the larval densities of *An. dirus, An. minimus* and *An. maculatus* (p<0.001), while there were no differences in *An. aconitus* and *An. barbirostris*.

Anopheles dirus has probably adapted to certain village environments by breeding in village domestic wells in Myanmar, in addition to its usual breeding sites in forested areas (Oo, 2003; Oo *et al*, 2004). High larval and pupal densities of *An. dirus* were found during the rainy season compared to the cool-dry season. Residential wells appear to have provided new breeding resources for *An. dirus* due to shortage of feral breeding sites as a result of deforestation (Oo, 2003; Oo *et al*, 2004) and aversion to sunlight. An alternative explanation is that they have only been noticed in wells after the forests have been cleared.

In Khanh Phu commune, *An. dirus* s.l. proliferated in artificial breeding containers, *eg* vases purposely placed along a transect at different distances ranging from 10 to 1,800 m from the houses into the forest. According to Marchand (2005), *An. dirus* "...*larvae were found throughout the year in the forest approximately 400-1,800 m from the inhabited areas and the average densities were not significantly different between the dry and wet seasons. Clear seasonal variations were seen especially in the zone from 200 to 400 m from the houses, a reforested area with high trees and closed canopy. In this zone An. dirus larval densities were much lower in the dry season, and twofold higher in the rainy season, compared with the corresponding deep forest densities. In the zone nearest to the habitations (10-200 m from the houses with open bushes), the larval density in rainy season was higher than in the dry one." These data from Khanh Phu site suggest that An. dirus has a stable base in the deep forest from which it is able to expand to the zones nearer to the village during the rainy season.*

1.1.4 Adult stages. Although the absence of the Dirus complex in large non-forested areas of Thailand, southern Viet Nam and central India is probably linked to the lack of suitable habitat(s), it has not been reported from the north of Viet Nam. This paradoxical situation is yet to be resolved, as northern Viet Nam is still forested and members of the complex are prevalent at the same latitude in neighboring GMS countries (Obsomer *et al*, 2007).

Apart from the association of *An. dirus* primarily responsible for forest transmission, Sanh *et al* (2008) and Marchand (2005) demonstrated active transmission in garden plots attended by villagers among the forested hills surrounding Dong Thong Village, Ninh Thuan





Fig 3–Trends in biting densities of *An. dirus* (per person per night) (A), entomological inoculation rates (EIR) (B) and annual malaria incidence (C) in Khanh Phu commune, central Highlands, Viet Nam. Data showing lower vector densities in 1999 to 2011 are not comparable with those from 2002 onwards as collections during the first three years were done in different types of forest (Marchand, personal communication).

Province and in plot huts and forests of Khanh Phu commune, Central Highlands (Fig 3), Viet Nam, respectively. Average densities of *An. dirus* were not different between rainy and rainless nights for any collection method (Marchand, 2005). Nocturnal rainfall did not influence the biting time, and peak activity times from 07:00 PM-01:00 AM were similar between rainy and dry nights. Moonlight increased the biting rate by 3.7 times more than moonless nights (Marchand, 2005).

When reared at 23°C, *An. dirus* and *An. sawadwongporni* were larger in body size, experienced prolonged development and produced a larger clutch of eggs relative to mosquitoes reared at 30°C. Temperature had no effect on egg hatching rate and sex ratio (Phasomkusolsil *et al*, 2011).

In two longitudinal one-year studies conducted in Sai Yok District, Kanchanaburi Province, Thailand, Sungvornyothin *et al* (2009) found that *An dirus* s.l. was more abundant during the wet season compared with the dry and hot seasons in Sai Yok District, Kanchanaburi Province, Thailand, while adult densities were positively associated with increased rainfall (July to August) (Tananchai *et al*, 2012a). This corroborates findings in Cambodia (Sochantha T, 2002, unpublished report) and in Viet Nam (Trung *et al*, 2004). In Thailand, vector abundance was positively correlated with increased rainfall and humidity, but was negatively associated with higher mean ambient temperatures.

Early biting was species and location-and temporal-dependent for *An. dirus* s.s. (= *An. dirus* A). The relative risk of being bitten in an hour before 10:00 PM by *An. dirus* s.s. in Lang Nhot (Khanh Hoa Province) was significantly higher than in Village 3 (Binh Thuan Province) and Cha Ong Chan (Rattanakiry Province, Cambodia) (Trung *et al*, 2005; Coosemans and Van Bortel, 2006; RBM, 2012). Blood feeding by *An. dirus* s.l. commenced immediately after sunset with a distinct peak activity at 10:00 PM in Thailand (Kanchanaburi Province) and Cambodia (Sochantha T, 2002, unpublished report).

Subtle differences in biting times were seen in two locations in Lao PDR. In Attapeu Province, *An. dirus* s.l. starts to bite as early as 07:00 PM but the peak is around 10:00 PM and biting continues throughout the night until 06:00 AM (Vythilingam *et al*, 2005a), whilst in Sekong Province, the peak biting times were around 09:00 PM to 00:00 PM followed by another smaller peak early around 03:00-04:00 AM (Vythilingam *et al*, 2003). At Pu Teuy village, Sai Yok District, Kanchanaburi Province of western Thailand, peak patterns of outdoor and indoor human-baited collections of *An. dirus* s.l. were similar, with a maximum landing density between 08:00 and 09:00 PM (Sungvornyothin *et al*, 2009). In this 2-year study, adult female mosquitoes were collected during 3 consecutive nights each month, beginning February 2005 to January 2007. Two years later, between September 2009 and August 2010,

and in the same village, *An., dirus* s.s.(molecularly identified) showed a prominent indoor biting peak between 07:00 and 08:00 PM and a smaller peak between 02:00 and 03:00 AM (Tananchai *et al*, 2012b) which is quite similar to the behavior in Sekong Province, Lao PDR. The outdoor human landing activity was elevated from 11:00 to 12:00 PM. In contrast, cattle baited collections showed one clear peak in the early evening (07:00-08:00 PM) followed by a decline throughout the rest of the night (Tananchai *et al*, 2012b); this pattern is similar to that observed by Sungvornyothin *et al* (2009).

The reason for the differences in feeding times and patterns compared to other sites in Thailand and other GMS countries is unclear, but it illustrates the biological variability (intraspecific and interspecific) between populations of the same or sibling species. Other possible explanations for the shift from 08:00-09:00 PM to 07:00-08:00 PM periods at Pu Teuy Village could be temporal variability and/or the vector's response to the irritability of the insecticide used by malaria control programs. It is feasible that the prolonged and widespread use of LLINs and IRS would favor individual survival traits such as biting outdoors or early in the evening as observed for *An. farauti* in Solomon Islands (Taylor, 1975). With time these traits may be selected for phenotypically and genetically, but so far there is inconclusive evidence for these evolving so as to avoid bednets (Pates and Curtis, 2005).

At Pu Teuy Village, a relatively larger number (54% of total catches) of *An. dirus* s.l. were consistently captured from the cow-baited collection compared to either human-bait collections, indicating that the feeding habits show a slightly greater preference for cattle (Sungvornyothin *et al*, 2009). In a follow-up study at the same village and using the same collection method, Tananchai *et al* (2012b) captured slightly higher numbers of *An. dirus* s.s. (molecularly identified) on cattle (63.2%) compared to 36.8% from indoor and outdoor landing collections, showing a relatively stronger zoophilic behavior. The use of AS-PCR technology to identify sibling species may account for this result as morphological identification would have produced an inaccurate analysis.

However, *An. dirus* s.s. showed a high degree of anthropophily from six geographically representative sites in Viet Nam (4 sites) and one each in Cambodia and Lao PDR (Trung *et al*, 2005). The ratio of outdoor human (OH) to cattle (OC) landing rates were 13.0, 35.17, 25/0, and 6/0 in Lang Nhot (Khanh Hoa Province, Viet Nam), Village 3 (Binh Thuan Province, Viet Nam), Cha Ong Chan (Rattanakiri Province, Cambodia) and Na Ang (Vientiane Province, Lao PDR), respectively (Trung *et al*, 2005). The difference between these findings and the Thai study may be explained by the use of different collection methods, seasons of the year and PCR analysis. The Vietnamese-Cambodia-Lao study used a cattle shed for collections from 09:00 to 12:00 PM each night for 10 nights in four (April, August, November 1998 and April 199) surveys in Viet Nam and three in Cambodia and Lao PDR (March, July

and October 1999). This methodology may have resulted in less than representative sample compared to the Thai studies which used system of rotating collectors between indoor and outdoor locations for the human landing collections; a separate team of two collectors for 15 minutes each hour for cattle baited collections, and the use of untreated cotton bednet for a cow. The same cow was used throughout the study and was placed at least 50 m from the nearest human landing collection site but at equal distances from the forest fringe to avoid a potential distance bias in attracting mosquitoes (Sungvornyothin *et al*, 2009; Tananchai *et al*, 2012b). In the follow-up study, PCR analysis revealed the presence of 58 specimens of *An. baimaii,* a sibling species in the Dirus complex which was hitherto unrecognized in the first study: 50% of these samples were captured on cattle, 39.7% were obtained from outdoor HLC and 10.35 from indoor HLC, compared to 63.2%, 28.1% and 8.7% for *An. dirus s.s.* respectively (Tananchai *et al*, 2012b). Well-designed entomological studies are needed to elucidate the role of sibling species in host preference using a combination of molecular identification method and a standardized mosquito collection protocol.

1.1.5 Response to control interventions

a) Insecticide treated bednets and other materials

Among the 13 ITN efficacy trials reviewed by Hosking (2010) in GMS from 1992 to 2010, ten indicated that ITNs were effective, one indicated that ITNs were ineffective and two trials were inconclusive. Of the eight trials conducted during 2003 to 2012, five indicated that ITNs were effective, one indicated that ITNs were ineffective and two trials gave ambivalent results.

Feeding time of vectors and human sleeping time are important determinants of the effect of ITNs in these studies (Pates and Curtis, 2005; Hosking, 2010). LLIHs and ITNs were effective for outdoor forest workers and early biting *A. dirus* s.l. in Cambodia (Sochantha *et al*, 2006, 2010), as well as for early to late night biters (09:00-00:00 PM) in Lao PDR (Vythilingam *et al*, 2003; Kobayashi *et al*, 2004), Thailand (Pates and Curtis, 2005) and Viet Nam (Erhart *et al*, 2004). Hosking pointed that the study performed by Smithius *et al* (2006) did not provide the necessary evidence to prove that ITNs were ineffective and generalizations made for the whole of Myanmar were inappropriate. As 51% of the mosquitoes (*An. sundaicus, An. maculatus, An. aconitus, An. subpictus, An. vagus*) fed before 08:00 PM this leaves 49% feeding by *An. annularis* after 08:00 PM (Hosking, 2010). *An. annularis* is considered a secondary vector in Myanmar (Table 1). Other data from Myanmar and from other GMS countries suggests approximately 60% of biting by *An. dirus* s.l. occurs before 10:00 PM (Networks, 2012). More local studies and entomological data in various ecotypes are needed to strengthen the evidence base for large scale promotion or deployment of ITNs in Myanmar as part of integrated malaria control efforts. Although no entomological data were available

in a forest malaria study by Erhart *et al* (2004) it was assumed that *An. dirus* s.s. was an early forest biter and that malaria was controlled by untreated nets that are used by people who regularly work in the forest but do not sleep there. LLIH seems to be effective and appropriate as their design offer better protection for forest workers in Viet Nam (Thang *et al*, 2009a) and Cambodia (Sochantha *et al*, 2010), see RESULTS section 1.1.5 (b).

The two ambivalent studies reviewed by Hosking (2010) were characterized by the occurrence of many different species with different biting habits suggesting that the effectiveness of ITNs will vary from place to place, see Chapter 7. There is therefore a need for more entomological studies to inform ITN distributions particularly in areas where they will be most effective.

After ITNs were introduced in Khanh Phu commune in Khanh Vinh District bordering the Central Highlands of Viet Nam, *An. minimus* s.s. virtually disappeared from 1998 and was replaced by *An. dirus* which continued to play an important role in maintaining malaria transmission in farm plots and forested areas (Fig 3; Marchand, 2005). Fig 3 reflects the situation in one community, but the situation may be very different in other GMS countries where intense transmission takes place in the village. In this situation, while some forest still remains on the surrounding hills no agricultural activities occur there. As these forests are only visited for transient activities such as timber cutting, hunting, gem mining and food gathering, the existence of forest malaria was corrected by Sanh *et al* (2008) who explained that the modified concept of forest malaria applies to persons who do not live in (or near) the forest but who visit it for various reasons and for varying periods.

In the forest and plothuts, 50% of *An. dirus* s.l. bites occurred before 10:00 PM compared to later biting in the villages (Marchand, 2005); similar data from Myanmar and other GMS countries suggests that overall approximately 60% of biting by *An. dirus* s.l. occurs before 10:00 PM (Networks, 2012). The time taken to fly from breeding and resting places in the forest to the villages during the rainy season is likely to account for the differences in biting times between the villages and forest/plot huts (Marchand *et al*, 2011). Partial coverage of ITN was associated with a significant shift in biting time in the villages from 46% to 65% before 10:00 PM. However, as biting densities were lower in the households with nets, the total risk of bites before 10:00 PM was still lower than in the untreated households (Marchand, 2005). The Khanh Phu case study highlighted the importance of the extended baseline phase from 1993 to 1997 during which critical information regarding the seasonal characteristics, malaria transmission systems and meteorology was available for evaluating the impact of ITNs. This point was emphasized by Hosking (2010) who recommended the collection of entomological data prior to large scale ITN distribution. For example, a joint malaria crossborder survey showed that LLIN/ITN on the Lao PDR side did not prevent *An. dirus* s.s.

mosquitoes from entering houses and be captured by indoor light traps, whereas no *An*. *dirus* were found on the Vietnamese side. Information on the actual utilization of bednets by people and the effectiveness of the insecticides used in reducing exposure to mosquito bites were not available (Pongvongsa *et al*, 2012). The high density of *An. dirus* in light traps in Laotian villages where most malaria infections were found compared to nil catches in the Vietnamese villages across the international border is a concern. The paucity of *An. dirus* is probably due to the absence of forest trees in Quang Tri, Viet Nam (<u>http://en.mapatlas.org/</u><u>Vietnam/Second-Order_Administrative_Division/Thi_Xa_Quang_Tri/6751/3D_earth_map/</u>) which may partly account for the lower malaria prevalence. Insufficient ITNs, (see RESULTS section1.1.5 e); (Hung, 2009), relatively late sleeping time (09:00-00:00 PM) and the slightly longer over night time in the forest may explain the significantly higher prevalence in Lao PDR.

In a mathematical model developed recently to inform containment operations, ITNs contribute to a 50% reduction in time to eradication of artemisinin-resistant *falciparum* malaria in western Cambodia; the model assumes a four-year effective life span of ITN and a 30% efficacy in reducing transmission (Maude *et al*, 2009). Thus ITN plays a supplementary role by providing a modest, sustained, protective effect which can have a significant impact on the time to elimination. Using a similar model fitted to the trial results from South West Cambodia, an optimum strategy for elimination was derived in which the addition of other interventions (mass drug administration and LLINs) further accelerated the process (Maude *et al*, 2012).

Cross-sectional surveys and follow-up of a passive case detection system conducted between March 2004 and December 2006 in a remote community with a high ITN (88.17%) and low hammock (13%) usage in central Viet Nam revealed substantial reduction of clinical cases and malaria infections by more than 50% and 70%, respectively; no controls were included in this study (Thang *et al*, 2009b).

In this setting of declining malaria incidence (Fig 3), forests that harbor *An. dirus* mosquitoes and macaque monkeys will provide a reservoir for the zoonotic transmission of *P. knowlesi* (see RESULTS section 1.1.1).

b) Long-lasting insecticidal hammocks

As discussed in RESULTS section 1.1.5, a significant proportion of *An. dirus* and other malaria vectors in GMS bite outside in the early evening before people go to sleep (Trung *et al*, 2005; Coosemans and Van Bortel, 2006). At the same time, Vietnamese or Cambodian villagers and forest workers rest in hammocks in the early evening. Long-lasting insecticidal hammock nets (LLIH) can, therefore, be a supplementary tool to prevent the bites of exophagic and early biting malaria vectors. Where forest activity is a very strong risk factor

for malaria infection, the traditional vector control methods are not effective in controlling forest malaria (Erhart *et al*, 2004, 2005; Ngo *et al*, 2008).

Using the same design of LLIH tested during the community-based intervention trial in central Viet Nam (Thang *et al*, 2009a), LLIH provided a significant reduction of *An. dirus* bites (46%; CI 95% 25-62) at the end of the rainy season in two villages of western Cambodia (Sochantha *et al*, 2010). This result probably underestimates the true protective efficacy of LLIH as volunteers sat in the hammocks the whole night with the nets open and their feet on the ground. The insecticide-treated flap of the LLIH was left open below the hammock to reduce landing of mosquitoes on the legs (Sochantha *et al*, 2010). This sitting position was chosen to simulate people's habit of spending the evening outside and to facilitate the collection of mosquitoes. Although not providing full protection, LLIHs could be effective in protecting forest workers and villagers during the pre-bedtime evening. A well-designed community-randomized trial similar to the LLIH evaluation study in an Amazon forested area where indigenous villages are scattered over a large territory (Magris *et al*, 2007) is needed in the region.

c) Durability of LLINs

Although pyrethroids are currently the only class of insecticide recommended for ITNs, it is still not clear to what extent this compromises their effectiveness against pyrethroid resistant mosquitoes, particularly once nets have acquired holes through wear and tear and therefore no longer provide a complete physical barrier. After five years of use in Lao PDR, the presence of large holes in polyester nets compared to Olyset Nets[™] suggest the latter are stronger (Tsuzuki et al, 2009), which is similar to a field evaluation of the same product after seven years of use in rural Tanzania (Tami et al, 2004). Polyethylene nets are more prone to fire damage according to anecdotal reports. The protective effectiveness of the LLIN does not dramatically deteriorate the year following the three year cut-off chosen by WHOPES for evaluation purposes although some acceleration of the decline was observed (Kilian et al, 2011). In addition to washing and physical handling, storage conditions affect the "useful life" or durability of a given LLIN product. The insecticide concentration and knockdown rate for three stored Olyset Nets[™] were low compared to the other nets routinely used in the houses. The LLINs were stored in the palm roof of the houses where daytime temperatures are very high (Tsuzukli et al, 2009). Heat and exposure to ultraviolet light can degrade permethrin (Gimnig et al, 2005; Sreehari et al, 2009).

A recent study conducted in experimental huts suggested that mosquitoes can pass through Olyset NetsTM despite high levels of insecticide if the nets have holes and/or are inappropriately used (Malima *et al*, 2008). Conventional deltamethrin bednets and LLINs

that were deliberately holed (six holes each 4 cm² were made in each bednet, two in each long side and one at each end, to simulate a torn net) acted as a barrier to *An. epiroticus* searching for blood meal, thereby demonstrating that the insecticidal treatment rather than the net, effectively prevented mosquitoes from entering the net and successfully feeding on the sleepers (Van Bortel *et al*, 2009).

Polyester nets treated with Mossmann 100 (permethrin 10%EC) at 300 mg/m² and recovered from the households were biologically effective against *An. dirus* s.l. for up to 6 months in the Pong Nam Ron District of Chantaburi Province, Thailand (Komalamisra *et al*, 2009). The observed mortality (67%) was above the recommended 50% cut-off recommended by Komalamisra *et al* (2009) for re-treatment of nets in areas where malaria is a perennial problem. The rapid decline in bio-efficacy was attributed to washing; however the washing conditions were not stated.

Owners of nets with larger holes (>20 cm²) have a high risk of malaria infection (OR 1.36; 95% CI 0.55-3.39) than owners of nets with smaller holes (0.25-19.9 cm²) (OR 0.87; 95% CI 0.32-2.35) or no holes (OR 0.88; 95% CI 0.39-2.02) in ethnic minority households in Binh Phuoc Province, Viet Nam (Abe et al, 2009). About 40% of the observed Olyset Nets[™] surveyed in Bourapar District, Lao PDR had holes/or were torn after 2-3 years of use (Shirayama et al, 2007); the mean number of holes in damaged nets was 2.3 (range: 1-22) and the mean area of the holes was 109 cm² (range: 0.8-3,000). Fifty-one respondents (21.3%) reported that they had found mosquitoes trapped inside nets. Hence it is important that malaria control programs implement monitoring and maintenance strategies for repair and replacement of holed or ineffective nets and promote the care of nets by their owners. Training residents to repair and store LLINs appropriately to avoid exposure to high temperatures and direct sunlight for long periods will ensure a maximum effective lifespan for nets. To overcome low literacy rates, maintenance instructions with illustrations are more appropriate than written texts. Questionnaire surveys showed that households who followed the recommendations and limited net washing frequency (38.2%) reported fewer malaria episodes during the previous year than those that did not, thereby demonstrating the importance of the recommended washing frequency in the effective use of LLINs (Shirayama et al, 2007).

As LLINs are increasingly used in GMS and have an average useful life of 3-5 years, durability studies are urgently required to determine their effective lifespan under real conditions, including the variation in performance between LLINs of different textiles, and the general environment in which the net is being used (climate, housing, sleeping place and washing patterns). Trials are hampered by the continuous development of new brands of LLINs and the procurement restrictions imposed by funding partners, *eg* Global Fund will not allow countries to specify polyester or polyethylene in the procurement process.

d) Behavior of LLIN/ITN users

The impact of ITNs is heavily reliant on significant behavioral changes among users to ensure the most vulnerable individuals sleep under the nets at night (Vanden Eng *et al*, 2010) and to maintain consistent compliance. Recent household surveys in Lao PDR showed that 89.6% of 240 households used bednets throughout the year and the rest used bednets seasonally or only sometimes in Bourapar District (Shirayama *et al*, 2007). Similar results were obtained in the recent 2011 MICS survey in Viet Nam [87.6% for children aged 0-59 months, 80.2% for pregnant women (expressed as percentage of children or pregnant women who slept under an ITN, living in households with at least one ITN; GSO, 2006, 2011], whereas, in Lao PDR, 86.7% of children under the age of five slept under any mosquito net on the night prior to the survey and 40.5% slept under an ITN (Department of Statistics and UNICEF, 2006). In the 2009 household surveys covering 8 provinces, 98.7% of 4,154 householders reported sleeping under a bednet the previous night (Hung, 2009).

Respondent-driven surveys on the Thai-Cambodia border showed >86% of Cambodian and Myanmar migrants slept under a bednet the previous night (Piyaporn *et al*, 2011). Household surveys indicate that >90% of households own a mosquito net in both Cambodia (NIPH, NIS and ORC Macro, 2006) and Viet Nam (GSO, 2006, 2011) although only 5% and 19%, respectively sleep under an ITN (WHO, 2010). Most of the respondents on both sides of the Lao PDR (83.3%) and Viet Nam (97.5%) border reported that they slept under a bed net during the previous night in the village proper compared to those who overnight in the fields (69.1% Lao PDR, 53.5% Viet Nam) (Pongvongsa *et al*, 2012).

High bednet utilization in Cambodia (85% in the Cambodian Malaria Survey 2010; Networks, 2012), Lao PDR and Viet Nam (see above; Thang *et al*, 2009b) is not surprising given that there is a strong "net culture" where they have been used traditionally for many years for privacy and protection against nuisance mosquitoes (see Chapter 7). Although bednet coverage has been increased in GMS countries (see Chapter 7), universal coverage is still a challenge especially in hard-to-reach areas (WHO WPRO, 2011). A household survey showed that there were still insufficient nets available for all occupants despite high ownership of bednets, *ie* 96.8% and 88.1% ownership of conventional bednets and ITNs, respectively (Hung, 2009).

e) ITNs in farm huts

Nonaka *et al* (2010) showed that overnight stays in farming huts was not associated with an increased risk of malaria infection in either the dry or rainy seasons in an endemic area in southern region of Lao PDR where ITNs were widely used both in permanent residences and farming huts. This finding suggests that previously reported association might be partly due to insufficient coverage or improper use of ITNs in the farming huts which have a higher risk of mosquito biting and transmission compared to villages (Fig 3). Overnight stays in farming huts are common in many GMS countries (Erhart *et al*, 2004, 2005; IOM, 2008; Ngo *et al*, 2008; Piyaporn *et al*, 2011) where both the adult population and children under five years of age travelled together to these places. Efforts should therefore be made to increase ITN coverage to protect everyone in these settings, as community-wide coverage is essential to suppress disease transmission and reduce exposure to unprotected persons (Maxwell *et al*, 2002; Hawley *et al*, 2003).

Sharing one net with up to three people in Viet Nam (Abe *et al*, 2009) or with five or more people in Lao PDR (Nonaka *et al*, 2010) significantly increased the odds of malaria infection risk. Almost all the nets in the Lao study were family size, which are usually designed to cover up to three children or two adults and an infant (Nonaka *et al*, 2010).

As forest huts are unlikely to be sprayed, occupants must also rely on ITNs for protection; however the floor area is sufficient for just one family-sized net. Insufficient numbers of nets may also force people to sleep together who would otherwise not. A higher prevalence of malaria infection was seen for beds with 4 to 6 people in Bin Phuoc Province (OR 2.11; 95% CI 1.12-3.98) compared to beds with 1-3 people (Abe *et al*, 2009). There are no reports regarding concerns about whether shared use of bed nets may be a factor in the emergence of sexual assault as a social and public health issue – by parent or relative against child, or by an older sibling against a younger one.

The Lao study suggests that people who practiced seasonal movements may need a greater number of nets than those who did not, even though the study respondents reported that they rarely stayed at the huts overnight (Nonaka *et al*, 2010). This may be explained by the habitual behavior to use nets for protection against daytime nuisance mosquitoes as was reported in a number of studies (Yoyannes *et a*, 2000; Adongo *et al*, 2005); and secondly, the difficulty involved with frequently carrying a net between the main residences and farming huts (WHO, 2003). Among 92.5% of forest-goers interviewed from 8 provinces in 2009, only 52.7% brought bednets with them to the forests; the most common reason cited for not purchasing bednets was the lack of pocket money (Hung, 2009).

A survey on the availability of bed nets in communities among 8 provinces in Lao PDR showed that 96.8% (4,132/ 4,270) families owned bed nets, "but the number of bed nets in households was low (less than 1 space under bed net/per person)" (Hung, 2009). "The proportion of the households have enough bed net for use was not adequate. A majority of people answered that they and their family used bed nets when sleeping (all people: 95.8%), and the rate of people sleeping under the bed net last night is 98.7% (4,098/4,154). The proportion of people not sleeping in bed nets said that the reason was economic (deficiency of bed nets) and a few people said that nets were not necessary or uncomfortable" (Hung, 2009).

There are insufficient bed nets per household to enable all members to sleep under cover.

Malaria program managers need to take into account extra nets used in farming huts or for mobile populations travelling to remote, hard-to-reach areas, when they decide how many ITNs are required for distribution.

As many farming huts are too small to accommodate the number of nets that would be mathematically calculated from coverage targets, there is probably a subjective element to assessment by community health staff who determine which family or household "needs" a replacement or supplementary ITN/LLIN. It almost certainly artificially reduces the denominator by which bednet coverage is calculated. More detailed studies of bednet utilization and community beliefs, attitudes and perceptions may be warranted, in addition to national surveys. This is important to enable understanding of how household or group decisions are made regarding the distribution of nets per family in the available sleeping space.

f) Indoor residual spraying

The literature on the impact of IRS in GMS countries is lacking due to near universal coverage with LLIN/ITN and very limited mass preventive or focal responsive spraying operations (see Chapter 7). However, IRS is considered as an effective measure for outbreak situations provided it is done in a timely and efficient manner and the operational and resource feasibility are considered in policy and programmatic decisions (WHO, 2011). Control of Anopheles vectors is a real challenge for the control programs due to varying exophagic and exophilic behaviors. The exophagic, exophilic and outdoor biting in combination with early feeding behavior of An. dirus s.s. (= dirus species A) will make both ITNs and IRS less suitable for controlling this species (Trung et al, 2005). Anopheles minimus s.l. and An. epiroticus are characterized as endophilic species; however Trung et al (2005) found them and all other anopheline species examined to be highly exophilic. Anopheles minimus s.s. exhibited a moderate degree of endophily and can therefore be controlled by IRS or ITN (see RESULTS section 1.2.3); but the large intraspecific behavioral differences, ranging from very anthropophilic and endophagic to exophilic and even zoophilic suggests that its role and amenability to control can differ from region to region (Van Bortel et al, 2004). Information regarding the efficacy of IRS on An. harrisoni (= minimus species C) is not available. The operational significance of exophily as measured during entomological surveys is probably overstated due to the limited number of study locations (three in Viet Nam and one each in Cambodia and Lao PDR), which are unrepresentative of the GMS countries. Under normal life, almost all villagers would be indoors from 10:00 PM and so a much higher proportion of man-vector contact probably occurs indoors than suggested by standard field trial data. Where entomological data are available, eq occurrence of 60% night-time biting before 10:00 PM, the relative risk of exposure is considerably higher in Lang Nhot, Viet Nam compared

to Cha Ong Chan, Cambodia and Village 3, Viet Nam (Trung *et al*, 2005; RBM, 2012). Opportunistic vectors will feed on people outdoors but if they are not available, mosquitoes will fly indoors and feed them there.

Experimental hut studies showed that both deltamethrin and DDT exerted strong excitatory responses on *An. dirus* s.I (Malaithong *et al*, 2010). Specifically, DDT appears to have a more pronounced and significant effect on behavior than deltamethrin, resulting in greater movement away from the insecticide source and thus potential reduction of bloodfeeding activity (Malaithong *et al*, 2010). However, these effects are not fully protective in local houses or farm huts built without walls or with incomplete walls that are sprayed with DDT compared to huts with complete walls, especially 6 months or more after spraying (as demonstrated in Sabah, East Malaysia (Cheng, 1968).

Excito-repellency tests demonstrated that three pyrethroids (bifenthrin, alpha-cypermethrin and lambdacyhalothrin) produced a rapid and pronounced irritant response in *An. dirus* s.l. without resulting in mortality following contact (Tananchai *et al*, 2012b). IRS with excito-repellent chemicals can create a paradoxical situation by causing an aversion to normal indoor biting behavior, while potentially reducing malaria transmission inside the houses, and increasing the probability for outdoor transmission.

As discussed in Chapter 7, there are risks in development of insecticide resistance associated with mass preventive IRS, and this could undermine the effectiveness of ITN campaigns where pyrethroids are used. Houses with open construction are not ideal for IRS as there are insufficient surfaces capable of retaining the residual insecticide. IRS requires systematic planning to cover areas with consistently high annual incidence ('mass preventive IRS') or confirmed foci of malaria transmission or outbreak areas ('focal responsive IRS'), consistency of the applied insecticide dose, and uniform coverage of sprayable surfaces.

A wider choice of insecticides including long-lasting formulations of lambda-cyhalothrin CS, deltamethrin WG, and pirimiphos-methyl CS (URL: <u>http://www.who.int/whopes/</u>) are now available for IRS programs, hence obviating repeated rounds of house spraying and minimizing fatigue and refusal by householders. IRS is applicable in new settlements in the new economic development zones with good road/railway networks or transportation facilities. Selection of insecticides for IRS needs to be guided by behavioral responses of local vectors to various chemicals, acceptability, efficacy and safety. Whilst a previous study have linked acceptance of IRS to a reduction in mosquitoes, nuisance biting, fulfillment of governmental orientations and group based citizenship (Montgomery *et al*, 2010; Munguambe *et al*, 2011), risk assessment must be made to ensure that the ecological balance is not intentionally disrupted by IRS. In Sabah, Malaysia, the increased damage made by moth caterpillars to thatch roof made of *nipa* (*Nypa fruticans* Wurmb) following DDT spraying was attributed to the fact that the insecticide killed the natural enemy of the caterpillar, the chalcid fly (Cheng, 1962). This small hymenopterous parasitic fly, *Antrocephalus* sp is a natural predator of the moth, *Herculia nigrivitta* Walker and comes into contact with the DDT whereas the caterpillar is not killed as the DDT does not reach the inner layers of thatch where it lives. However, malathion spraying apparently killed both caterpillar and chalcid, thereby prolonging the life of the roof (Thevasagayam *et al*, 1978). In the neighboring state of Sarawak, Malaysia, thatch damage was not an issue as *bilian*, an extremely hard wood resistant to moth larvae, was used for roofing (Chen and Chen, 2009). Whilst it is necessary to ensure that occupants of farm huts adhere to IRS, efforts must be made to safeguard the natural ecosystem and to avoid unintended consequences.

g) Insecticide-treated plastic sheeting

Insecticide-treated plastic sheeting (ITPS) is a promising alternative vector control to provide protection against endophilic vectors and the entomological effectiveness of deltamethrinincorporated ITPS has been demonstrated under a range of field conditions (Stiles-Ocran et al, 2010; Mittal et al, 2011; Pulford et al, 2012). In view of the widely recognized logistical constraints associated with spraying campaigns, ITPS or durable lining (DL), fixed to interior walls and/or ceilings offers a long-lasting alternative to IRS. DL is currently manufactured commercially (ZeroVector[®], Vestergaard Frandsen, Switzerland) as a thin sheet of woven high-density polyethylene (HDPE) shade cloth, which has insecticide incorporated during production; it is designed to cover interior walls and remain efficacious for three to four years. ZeroVector® DL is based on long-lasting net technology where deltamethrin is incorporated into the polymer before yarn extrusion, allowing it to migrate to the surface in a controlled fashion and ensuring uniform coverage, regardless of surface texture or wall shape. A multicenter trial was conducted in five African countries and Viet Nam to assess the feasibility, durability, bioefficacy and household acceptability of DL, compared to conventional IRS or insecticide treated curtains in a variety of operational settings (Messenger et al, 2012). The evaluation conducted in six houses in two rural villages in Hoa Binh Province, Ky Son District, Phuc Tien commune, northern Viet Nam showed a high acceptability among rural inhabitants which identifies them as the ideal target consumer group for DL. DL remained fully efficacious against pyrethroid-susceptible An. dirus (Khanh Phu), demonstrated minimal loss of insecticide content over 12 months of field use, and was unequivocally more popular than IRS and other long-lasting vector control products. Unfortunately, currently available DLs are pyrethroid based and may not represent a viable long-lasting alternative to IRS due to their capacity to exert selection pressure for insecticide resistance.

h) Barrier spraying and livestock sponging

While the control of An. dirus and forest malaria is a significant problem for GMS, there

are still many rural areas where, though the land has been cleared for cultivation and *An. dirus* is relatively uncommon, malaria still persists. In deforested areas and rural communities in north cental Viet Nam, malaria transmission was maintained by a number of anopheline species (comprising >80% of *An. sinensis, An. aconitus, An. harrisoni, An. maculatus, An. sawadwongporni* and *An. philippinensis*) which feed predominantly on domestic animals and only secondarily on humans (Manh *et al*, 2010). Their differing preferences for oviposition sites over the year allowed for low level but persistent year round malaria transmission, which more or less reflect the findings in GMS countries (Harbach *et al*, 1987; Rattanarithikul *et al*, 1996; Coleman *et al*, 2002; Trung *et al*, 2004). The attractiveness of cattle and buffalo tends to concentrate the mosquitoes into small and well defined areas within the village and as the host seeking mosquitoes rest on vegetation, in the immediate vicinity of the animal host both before and after feeding, barrier spraying may have a greater impact than IRS and LLINs (Manh *et al*, 2010). Although barrier spraying of vegetation has been used in various situations against a number of pest and vector species (Perich *et al*, 1993; Amoo *et al*, 2008) there is a lack of studies on the effectiveness of this intervention in local settings.

Another strategy is the treatment of pyrethroids on the surfaces of domestic livestock (applied by a sponging method) in the expectation they would act as toxic bait. Entomological trials conducted in Pakistan showed that deltamethrin was effective in killing mosquitoes for up to 4 weeks after treating cattle (Hewitt and Rowland, 1999). A community-randomized trial in which refugee communities sponged their domestic animals with deltamethrin four times a year reduced the incidence of *P. falciparum* malaria by 56% (CI 14-78; Rowland *et al*, 2001). IRS of houses in the same group of camps in previous years used five times as much insecticide for about the same impact (Rowland *et al*, 1997; Rowland and Nosten, 2001).

1.1.6 Behavioral responses to insecticides. Since the withdrawal of DDT for IRS in 1994 in Thailand (Patipong, 2000; Malaithong *et al*, 2011) and other GMS countries due to unsubstantiated environmental pollution concerns, various synthetic long-lasting formulations of insecticides have been accepted for use in re-treatment of bednets (*eg*, deltamethrin in KOTab-123[®], and lambdacyhalothrin in ICONMaxx[®]) and for IRS (*eg*, pirimiphos-methyl CS, lambdacyhalothrin CS, and deltamethrin WG). The continuing wide-scale use of pyrethroids is a major stimulus for continuing studies on the effectiveness and impact on malaria transmission and on the behavioral responses of *Anopheles* populations. Behavioral responses to insecticides can generally be classified into at least two distinct categories: contact stimulation ("excitation, irritancy") and noncontact spatial repellency ("deterrency").

Behavioral outcome studies have been documented with the *An. dirus* complex and various other *Anopheles* species as well as culicine mosquitoes using the excito-repellency test chamber (Chareonviriyaphap *et al*, 2004; Polsomboon *et al*, 2008a; Tananchai *et al*,

2012b). As pyrethroid-class insecticides are known to elicit excito-repellent responses in mosquitoes, assays with these test chambers can provide an overall assessment of an insecticide's ability to control disease transmission. A susceptible colony of An. dirus species B from Thailand demonstrated the weakest response to deltamethrin but strong repellency was observed in a field population of An. dirus s.l. with more than 50% of the test populations escaping from the test chambers within 30 minutes (Chareonviriyaphap et al, 2004). Mortality was low in mosquitoes escaping the treated chambers in contact and noncontact trials, an indication that behavioral avoidance greatly reduces the opportunity for residual insecticides to impact survival through toxicity. Observations of higher numbers of escaped An. dirus species B colony mosquitoes compared to other test populations may be explained by prolonged colonization following 16 years of continuous maintenance in the laboratory and isolation from varying natural stimuli (Chareonviriyaphap et al, 2004). Excito-repellency assays using wild caught An. dirus s.l. exposed to operational field dose of three synthetic pyrethroids and DEET (N, N-Diethylmeta-toluamide) show that the mosquitoes exhibits both irritant and repellent actions (Tananchai et al, 2012b). Alpha-cypermethrin demonstrated the strongest irritant action (89.4% escape), followed by DEET (77.0%), lambdacyhalotrhin (68.6%) and bifenthrin (68.3%).

The behavioral avoidance of treated surfaces, especially irritancy as observed in these experiments generally prevents sufficient contact with a residual insecticide, thus greatly reducing the risk of premature mortality in blood-seeking mosquitoes (Chareonviriyaphap *et al*, 2004; Tananchai *et al*, 2012b). However, Roberts *et al* (2000) showed that a reduction in the toxic effects of a chemical may not necessarily mean an increase in risk of human-vector contact inside houses. The Thai studies provide a convincing argument that the consequence of the combined effect of repellency and irritancy in reducing house-entering mosquito densities and interrupting blood feeding behavior exerts a profound influence on transmission which is likely to override the influence of contact toxicity.

With *An.cracens* and *An. scanloni*, contact irritancy and noncontact repellency were quite high, especially at the operational doses. As most of the work on the excitation and repellency of mosquitoes to insecticides were conducted under laboratory-controlled conditions using an excito-repellency test system (see above), it is difficult to extrapolate these bioassay data on insecticidal effects (whether toxicity or excitation) and degree of protection against mosquito blood feeding inside a house or community-wide setting. Therefore, experimental hut trials designed to provide more direct evidence on the true biological and epidemiological impact, showed that *An. dirus* were 50% less likely to land on humans inside a DDT-treated hut compared with the deltamethrin-treated hut (Malaithong *et al*, 2010). Although both chemicals exerted strong excitatory responses, DDT appears to have a more pronounced

and significant effect (p=0.002) on behavior than deltamethrin, resulting in greater movement away from the insecticide source and thus potential reduction of blood-feeding activity. The variation in the landing patterns of *An. dirus* suggests deliberate avoidance of DDT-treated surfaces primarily through spatial repellency and deltamethrin treated surfaces by contact excitation (irritancy) (Malaithong *et al*, 2010).

1.1.7 Response to botanicals and plant extracts. Using a field-automated excito-repellency test system, field collected *An. harrisoni* exhibited a higher escape response at 2.5% catnip oil (*Nepeta cataria* L.) from the contact chamber, while in the non-contact chamber a higher escape response was observed at a concentration of 5% (Polsomboon *et al*, 2008a). Although the protection time was not evaluated, catnip oil has strong irritant and repellent actions on mosquito test populations as indicated by the comparatively low escape time.

Among the six promising larvicidal plants (*Rhinacanthus nasutus* (L.) Kurz, *Derris elliptica* (Wallich) Benth, *Trigonostemon reidiodes* (Kurz) Craib, *Homanlomena aromatica* (Sprengel) Schott, *Stemona tuberosa* and Lour and *Acorus callamus* L., the petroleum extract of *R. nasutus* roots (Thai name: *thong phan chang*) exhibited high larvicidal effects against insectary colonies of *An. dirus* larvae with LC_{50} values of 7.91 mg/l (95% CI 5.30-11.73 mg/l) compared to LC_{50} of 14.51 mg/l (95% CI 13.13-16.02 mg/l) from methanol extracts of the same plant (Komalamisra *et al*, 2005). Similar effects on *Ae. aegypti, Mansonia uniformis* Theobald and *Culex quinquefasciatus* Say were also reported. The high larvicidal activity of *R. nasutus* and *D. elliptica* and the abundance of these plants in tropical and subtropical countries may be a cost-effective opportunity for field use in mosquito control programs. Although the operational and technical feasibility of larviciding against *An. dirus* and forest malaria vectors has not been tested, it is definite that this intervention will not be effective for this species.

1.1.8 Insecticide resistance. Insecticide resistance poses a major potential threat to the enormous malaria control efforts which have resulted in a significant decrease in the malaria burden in GMS (Ettling, 2002; Barat, 2006; WHO, 2010, 2011a). DDT use in Sri Lanka had dramatic effects in reducing malaria mortality in the 1950s until the 1970s when high levels of DDT resistance required a complete substitution by organophosphates and pyrethroids. Because of the higher costs of these insecticides,the long continued civil war in the north of the island and incomplete spray coverage, malaria incidence increased from 17 cases in 1963 to 360,000 cases in 1994 (Curtis, 2002). IRS with DDT was quickly reintroduced but, due to emerging DDT resistance did little to stem a major epidemic in 1967-1968 (Ministry of Health Sri Lanka, 2012).

Insecticide resistance status of the major malaria vectors in Cambodia, Lao PDR, Thai-

land and Viet Nam was assessed by a monitoring network called MALVECASIA for the first time from 2003 till 2005 (Van Bortel *et al*, 2008). The specific locations where resistance was found for each species are published (Van Bortel *et al*, 2008). The MALVECASIA database of 100 sites provides content information regarding 30 different *Anopheles* species and 12 to 20 different species were collected in various local scale surveys in Cambodia and Vietnam, (Obsomer, 2010). The species most tested were *An. minimus* s.l. (37.8%), other *Anopheles* species (*An. vagus, An. sinensis, An. paraliae, An. peditaeniatus*) (34%), *An. dirus* (13.06%), *An. epiroticus* (7.86%), *An. maculatus* (5.24%), and *An. campestris*-like (1.97%) mosquitoes.

One *An. dirus* s.s. population was resistant against lambdacyhalothrin, with a mortality of 75% tested on 66 specimens from central Viet Nam. Regionally, *An. dirus* s.s. was susceptible to permethrin (Van Bortel *et al*, 2010) and no *kdr* mutation (Verhaeghen *et al*, 2009; Cui *et al*, 2012) (Knockdown resistance kdr is a well-characterized mechanism of resistance to pyrethroid insecticides in many insect species and is caused by point mutations of the pyrethroid target site the para-type sodium channel) was observed in Cambodia, Lao PDR, Thailand and Viet Nam. Thus, true resistance was limited to *An. epiroticus* (= *An. sundaicus* A) (see RESULTS section 1.3.5), which is a secondary vector in coastal regions, and *An. vagus* which may be a minor vector. Data on insecticide susceptibility from Myanmar is lacking.

Larvae of *An. cracens* (= *An. dirus* species B, Thailand colony) were susceptible to pyriproxyfen; at 0.05 ppb, males exhibited significantly higher susceptibility than females (Satho *et al*, 2003).

A study to identify risk areas for the development of insecticide resistance as a consequence of crop protection activities in agriculture showed high insecticide use in vegetables and fruits in Thailand (Overgaard, 2006). Small and scattered areas where vector resistance might develop through exposure to agricultural insecticides, apart from some larger, relatively contiguous areas in northern Chiang Mai, were also identified. Reduced susceptibility to methyl parathion, an agrochemical in An. maculatus populations in a location with intense agricultural intensity in northern Thailand was caused by intensive agricultural pest control and not be vector control activities (Overgaard et al, 2005). It seems reasonable to extrapolate these findings to the rest of the GMS as increased use of agricultural insecticides and pyrethroids will inevitably contribute to the rise of resistant mosquitoes and may cause problems for future vector control. In Africa, agriculture has been an important prompt for the initial appearance of resistance in some localities, but the massive scaling up of LLINs and IRS for malaria control has been the main factor driving the recent increases in the geographic distribution and frequency of insecticide resistance genes in malaria vectors (WHO, 2012). WHO (2012) and Overgaard (2006) recommended collaboration between the agricultural and health sectors to improve resistance surveillance and to initiate integrated pest and
vector management intervention to avoid or minimize double insecticide exposure to insect vectors and to reduce risks to human and environmental health.

Chareonviriyaphap *et al* (2000) noted that the development of insecticide resistance in mosquitoes in Thailand has been very limited despite long-term use of chemicals for malaria vector control. This may be explained by the exophilic and exophagic behavior of *An. dirus* and significant behavioral avoidance responses which contributed to effective reduction of human-vector contact than toxicity. Various studies on the responses of vector populations from different geographical locations in Thailand have confirmed this behavior (see RESULTS sections 1.1.6 and 1.2.4).

There are several factors affecting the epidemiological and operational impact of insecticide resistance (WHO, 2012). First, in some cases the strong expression of metabolic resistance occurs in young mosquitoes, but, when they aged, susceptibility returns. Therefore, insecticide still kills older mosquitoes (Lines and Nassor, 1991). Second, the ability of resistant vectors to transmit malaria may be reduced as, even if they are not killed by an insecticide within 24 hours, the insecticide may still inhibit their ability to live for the 12 days necessary to become infective. Third, the physical barrier of ITNs provides a degree of protection, even if the insecticide is no longer effective, whereas IRS does not have this physical protection effect. Fourth, reduced effectiveness is initially seen in places where a higher threshold of resistance occurs, whereas control failure may occur only after the strength and frequency of resistance have increased. Fifth, vectors are still killed because they come into contact with an insecticide on many occasions within a short time (Hodjati and Curtis, 1999). However, resistant An. minimus have a higher excito-repellent response when exposed to deltamethrin (see RESULTS section 1.2.4), and may not remain sufficiently exposed to the insecticide or receive a larger dose and be killed. With the exception of the first factor, the other issues are hypothetical scenarios.

Although the resistance situation in GMS is not as serious as in sub-Saharan Africa and India due to the combination of widespread reports of resistance – in some areas to all classes of insecticides – and high levels of malaria transmission, GMS countries have not monitored resistance as comprehensively as required (WHO WPRO, 2012). Coverage of representative sentinel sites is limited or rarely monitored consistently over time. In addition, they may have no system for efficient analysis and reporting of data. This may result in pockets of undetected foci of resistance or higher levels of resistance may have reached in hitherto unreported areas where the number of malaria cases has increased.

Noting that monitoring of insecticide resistance is less systematic than before in the GMS (AusAID, 2012), WHO, PMI and partners are currently implementing the Asia Pacific Network for Vector Resistance (APNVR) to ensure more regular monitoring (APMEN, 2012; WHO

WPRO, 2012). Malaria control programs should introduce random spot checks to supplement sentinel site surveillance especially in areas where control efforts appear to be failing.

1.2 Minimus complex

The Minimus complex belongs to the Minimus Subgroup under the Funestus Group of the Myzomyia Series, Anopheles (Cellia) (Table 2). The Funestus Group includes about 21 species in Asia and Africa, of which 6 species are found in the GMS. The Minimus complex comprises of three species, namely An. minimus Theobald (= An. minimus A), An. harrisoni Harbach and Manguin (= An. minimus C), and An. yaeyamensis Somboon and Harbach (= An. minimus species E) (Harbach et al, 2006, 2007; Sinka et al, 2011), with only the first two species known to occur in the GMS. Differences in phenology between An. minimus and An.harrisoni have been noted in Thailand and Viet Nam (Garros et al, 2006). Anopheles minimus s.l. is considered a primary malaria vector taxon in the hilly forested regions in the Oriental Region that extends northwards to about 32° 30' N in China, westward to Uttar Pradesh in India, southward to the Thai-Malay Peninsula (Manguin et al, 2008b), possibly reaching south to the northwestern corner of Malaysia (Reid, 1968), and eastward to the Ryukyu Archipelago of Japan (Sinka et al, 2011). The geographical distribution of An. minimus based on expert opinion and known occurrence records is shown in Fig 2B. Vectorial status is uncertain, although An. minimus seems to be a more efficient malaria vector than An. harrisoni (Trung et al, 2004; Garros et al, 2005b). Anopheles yaeyamensis is restricted to the Ryukyu Archipelago in southern Japan where it was the principal vector before malaria was eradicated in 1962 (Sinka et al, 2011). Chen et al (2002) reported An. harrisoni in southern China up to 32.5°N; however, An. minimus and An. harrisoni are known to be sympatric across large areas of southern China, Thailand, northern Viet Nam and northern Lao PDR (Chen et al, 2002; Garros et al, 2006, 2008; Manguin et al, 2008b). In Thailand, An. minimus occurs throughout the country while An. harrisoni is confined in the western and northern subregions, including Tak and Chiang Mai Provinces (Rattanarithikul et al, 2006). Foley et al (2008) noted that the two species overlap broadly in ecological space in Asia, with An. minimus ranging more broadly into hotter and wetter climates of the tropical areas of Indochina, whereas that for An. harrisoni includes cooler and drier climates of the more temperate areas of China. Differences in the elevational occurrences of the An. minimus complex may reflect differences in the species composition in these regions. Harrison (1980) noted that An. minimus s.l. immatures were not collected over 672 m above sea level (asl) in Nepal (Pant et al, 1962), but they were found up to 1,500 m in Viet Nam (Lysenko and Ngi, 1965). Based on ecological niche modeling results and observations in the literature, the Nepalese records probably refer to An. minimus, whereas either species is possible for Viet Nam (Foley et al, 2008). Table 2 includes the distribution of the species in the Minimus complex and other

members of the Funestus Group.

Molecular tools were developed to identify all the species belonging to the Minimus complex that are present in the region, *eg* the allele-specific-PCR assay is more frequently used to distinguish *An. minimus* and *An. harrisoni*, due to its reliability, rapid and easy one-step PCR application (Garros *et al*, 2004a, b, 2005a, c; Phuc *et al*, 2003; Manguin *et al*, 2008b).

Misidentification of *An. minimus* s.s is problematic for public health personnel conducting vector control due to the high morphological variability and habitat similarities with other members of the Myzomyia Series, particularly Aconitus Subgroup (*An. aconitus* Donitz, *An. pampanai* Buttiker and Beales, and *An. varuna* lyengar). Furthermore *An. minimus* and *An. harrisoni* are sympatric and exhibit specific behaviors and vectorial roles, and it is important to differentiate them. Analysis of nine populations throughout Southeast Asia and from two sites in Thailand showed that the wing patterns present spatial and temporal variation that make these morphological characters unreliable for the precise identification of *An. minimus* and *An. harrisoni* (Sungvornyothrin *et al*, 2006a). Similar conclusions regarding the unreliability of the humeral pale spot for sibling species differentiation were made by Cuong *et al* (2008). PCR-restriction fragment length polymorphism (RFLP) of ribosomal DNA ITS2 successfully confirmed the identification of *An. minimus* s.s. in 8 morphological groups collected from Mae Sot district, Tak Province, Thailand (Jaichapor *et al*, 2005) highlighting the importance of molecular identification for unambiguous differentiation.

Phylogenetic analysis using D3 and ITS2 regions among populations of the *An. minimus* complex revealed three groups: the Japanese population as group 1, the population from Guangxi Province of China as group II and others, as group III in which *An. minimus* A is included (Thailand and Indonesia) (Sawabe *et al*, 2003). Populations of *An. minimus* that were examined using 144 mtDNA COII sequences from 23 sites in China, Thailand and Viet Nam showed high haplotype diversity, with two distinct lineages that have a sequence divergence of over 2% and exhibit different geographical distributions (Chen *et al*, 2011). This observation may be explained by a model of past fragmentation into eastern and western refugia, followed by growth and range expansion, and is consistent with the palaeo-environmental reconstructions currently available for the region.

1.2.1 Bionomics and susceptibility to *P. vivax* and densovirus. Whilst the distribution and abundance of *An. dirus* s.l. have decreased in eastern Thailand at the border of Cambodia, the abundance of members of the *An. barbirostris* Group (= *An. barbirostris/campestris*) have increased (Limrat *et al*, 2001; Overgaard, 2006). This may be explained by changes in vector potential, *ie*, changes in the composition and abundance of vectors that have a high affinity to transmit *P. vivax* (the relative abundance of which has increased from <20%

in 1965 to >50% in 2002) (Sattabongkot *et al*, 2004a). In northwestern Thailand, the EIRs of *An. minimus* for *P. vivax* during the wet and dry seasons were 0.023 and 0.010, respectively compared to 0.005 for *P. falciparum* in the wet season, showing a 4.6 fold difference (Sithiprasasna *et al*, 2003). Increased natural infections of Pv phenotypes VK210 and VK247 were observed in *An. minimus* (5 positive for VK210, 3 positive for Pf) (Coleman *et al*, 2002). *Anopheles pampanai* was for the first time confirmed as a vector of *P. vivax* in Viet Nam (Durnez *et al*, 2011).

The first experimental infection of *An. minimus* s.l. with the Thai-strain densovirus, *ATh*DNV showed the lack of significant larval mortality in the low virus concentration (9.5%) and controls (7.5%) with vertical transmission rates ranging from 25.0-53.8% (Rwegoshora *et al*, 2004). Although densovirus did not appear to influence *An. minimus* fecundity, in terms of number of eggs laid or hatched, the pathogenecity of *ATh*DNV, particularly at high virus concentration may provide a possible biological control tool.

1.2.2 Immature stages. Anopheles minimus s.l. immatures occur principally in stream pools and stream margins, with cool unpolluted water with partial shade and grassy margins. Larvae are also found in ponds, lakes, palm swamps, seepage pools and springs, rock pools, small ditches, bogs and marshes, ground pools, and rice fields (including fallow fields and pools in dry fields) (Rattanaritikhul et al, 2006; WRBU, 2012). In the suburbs of Hanoi, Viet Nam, unusual larval habitats for An. minimus include rain water tanks. In northern Viet Nam and western Thailand, An. minimus habitats vary from dense canopy forest to open agricultural fields, particularly traditional rice agro-ecosystems. Using remote sensing GIS and LANDSAT satellite data, Rongnoparut et al (2005) showed that An. minimus s.s. has a wide habitat preference, from dense canopy forest to open agricultural fields, while An. minimus C has a narrow habitat preference. In the Thai-Myanmar border area, light forests were defined as areas covered by trees that are accessible to people. Multivariate analysis revealed that light forest cover was a significant factor only for *P. falciparum*, and the mean distance from an individual house to a stream was about 200 m with a range of 200 to 500 m (Lawpoolsri et al, 2010). This relatively small variation suggests that vector abundance is similar among houses. The risk of Pf attack among people in a hamlet increased by 1.03 for every 1% increase in the hamlet's light forest cover but this association is uncertain due to the limited number of landscape data for comparison at hamlet-level (Lawpoolsri et al, 2010).

Using IKONOS satellite imagery and Global Positioning System to map overall malaria cases and major vector breeding habitats, Sithiprasasna *et al* (2005) could not find significant difference between the proximity to streams between houses with and without malaria cases. This may be explained by the diverse landscape being dominated by forest to corn

land-use and orchard plantations and restricting the proximity analysis within a 1.5 km buffer from the breeding sites which was less than the suspected flight range of *An. minimus* s.l., *ie*, >1.5 km. The odds of finding *An. minimus* larvae were significantly higher for habitats located in orchards and villages than in forests (Vanwambeke *et al*, 2007a). Given the lack of association between forest cover and malaria risk, further research with wider spatial and temporal scale is needed to understand which environments might favor transmission.

The presence of *An. minimus* immatures was associated with flowing drainage, wet season, high pH and aluminum levels (Kankaew *et al*, 2005). They were found in flowing drainage 35.0 times higher than in non-flowing drainage, 0.4 times higher in the wet season than in dry season, while in alkaline (pH 7.5+) soil 5.4 times higher than acidic soil (pH <6.5), and 0.3 times higher at aluminum levels 80-125 ppm than at 5-10 ppm. Only flowing drainage (*eg*, stream margins and stream pools) was found to be a parameter associated with the presence of *An. minimus* (Kankaew *et al*, 2005). The higher requirement of *An. minimus* for water hardness is probably responsible for its abundance. Lower temperature and carbon dioxide concentrations in the breeding sites contributed to an increase in population density (Kengluecha *et al*, 2005a, b).

1.2.3 Adult stages. Anopheles minimus s.s. and *An. harrisoni* are considered vectors of malaria parasites throughout their respective distributions, but *An. minimus* appears to be the more important vector. *Anopheles minimus* s.l. is considered a primary malaria vector in the hilly forested regions of mainland Southeast Asia. Sporozoites rates between 0.3-1% were recorded in Thailand in 1990, but no infection was observed along the Thai-Cambodia border in 1988 (Meek, 1988; Gingrich *et al*, 1990; Manguin *et al*, 2008a).

Prior to IRS and ITN implementation in Khanh Phu commune, Central Highlands, Viet Nam, 98% of *An. minimus* s.l. were recaptured within a radius of 600 m in mark-recapture studies (Marchand, 2005), whereas Tsuda *et al* (2011) recorded an average flight distance of 251 m, with a few individuals reaching up to 2,700 m from the release point. This observation was consistent with an ellipsoid spatial analysis which showed that the maximum average distance from the patient's house to vector breeding sites was greater than 1.5 km (Sithiprasasna *et al*, 2005) or 3 km (Lek-Uthai *et al*, 2010). In the Thai-Myanmar border, the variation of mean distance from an individual house to a stream was relatively small, about 200 m with a range of 200 to 500 m, suggesting that vector abundance is similar among houses within this range (Lawpoolsri *et al*, 2010).

This species was mostly found resting indoors at a height of 0.5 to 1.6 m above the floor and at a temperature and humidity range of 23-28°C and 61-88% RH, respectively (Marchand, 2005). Nocturnal rainfall did not influence the biting of *An. minimus* in Khanh

Phu commune, although the average densities were much higher in the dry nights than in rainy nights, except for outdoor human bait (very few cases) (Marchand, 2005). Moonlight increased the biting rate of *An. minimus* by 1.4x, but did not significantly change the peak biting time.

Using octenol dehydrogenase as a genetic marker to identify An minimus s.s. (= An. minimus species A) and An. harrisoni (as species A and C, respectively), Van Bortel et al (2004) demonstrated large intraspecific differences among populations of An. minimus s.s. This heterogeneity is illustrated in the ranking of anthropophily expressed as outdoor human/outdoor cattle ratios as follows: 0.10 (Hoa Binh, northern Viet Nam), 8.10 (Lang Nhot, south-central Viet Nam), 0.47 (Cambodia) and 10.11 (Lao PDR) (Trung et al, 2005). A more anthropophilic feeding behavior and preference to blood feed outdoors (approx. 60%) by An. minimus s.s. (confirmed by multiplex AS-PCR) was observed in Mae Sot District, western Thailand (Tisgratog et al, 2012). In northern Viet Nam An. minimus s.s. adults were more zoophilic compared to south central Viet Nam, Cambodia and Lao PDR where they showed marked anthropophilic behavior when cattle were scarce. In the most northern study site, An. minimus s.s. showed noteworthy endophilic behavior. As An. harrisoni was only found in one locality, intraspecific behavioral variation was not assessed. It was primarily zoophilic and therefore, its vectorial status is questionable (Van Bortel et al, 2004). As An. minimus s.s. is able to change its host preference in function of local situations in host availability, its role in malaria transmission can vary from region to region. Consequently, the impact of vector control on this species may differ between localities.

Seasonal variation studies of *An. minimus* s.l showed the peak biting behavior during the months of November and December where the majority of biting occurred before 10:00 PM (Sochantha, 2002 unpublished report; Cooke, 2003; Cooke and Vanne, 2003). Nocturnal adult feeding patterns of *An. minimus* s.l. in Kanchanaburi Province, western Thailand were similar during the wet and dry/hot seasons, with two distinct peaks: a strong activity immediately after sunset (06:00-09:00 PM) followed by a second, less pronounced, rise before sunrise (03:00-06:00 AM) (Chareonviriyaphap *et al*, 2003a). This may be explained by the presence of *An. harrisoni* with an all-night outdoor feeding activity and a distinct feeding peak immediately after sunset (06:00 PM) whereas the indoor feeding showed two small peaks at 08:00 PM and 12:00 PM (Sungvornyothin *et al*, 2006b). The difference in human-biting peak of *An. minimus* in other localities in Thailand is indicative of mixture of sympatric sibling species or intra-specific variation due to site-specific populations exhibiting different host-seeking behavior.

1.2.4 Response to control interventions. In Cambodia where *An. minimus* s.l. was more prolific, the *Anopheles* catches showed no significant difference between the proportion of

mosquitoes active during hours when the population would be protected by ITNs and those hours when they would be exposed to potential infective bites. Cooke (2003) estimated that 46% of potential malaria vectors were active during the period when people were unprotected, *ie*, 06:30-09:30 PM and 03:00-06:30 AM, indicating that ITNs could not be relied on to offer complete protection from infection at the time of the study in July 2003.

Retrospective analysis of the effects of ITNs revealed that *An. minimus* s.s. (= *An. minimus* species A) was effectively controlled by ITN as the field observations confirmed the hypothesis that this species was caught in the rainy season in the plot huts and was predominantly outdoor biting (Marchand, 2005). After the disappearance of *An. minimus* s.s. (see RESULTS section 1.1.5 a), malaria transmission was significantly reduced in the villages and increased in farm plots and forest locations (Fig 3), with only one transmission season at the end of the rainy season to early dry season. On the other hand, *An. minimus* s.l. (and *An. maculatus* complex) has increased significantly in density in the last decade especially in forested areas in Mae Hong Son Province, Thailand (see RESULTS section 3; Suwonkerd *et al*, 2004). These species are mainly observed in the wet season and may have contributed to the higher malaria incidence in the first peak from recent observations (Childs *et al*, 2006).

Entomological field studies in western Cambodia showed that personal protection conferred by long-lasting insecticidal hammocks (LLIHs) was 46% (CI 95% 35-55%) against the bites of *An. minimus* (Sochantha *et al*, 2010; also see RESULTS section 1.1.5 b). Excitorepellency tests (see RESULTS section 1.1.6) showed that contact irritancy was a major behavioral response of both *An. minimus* and *An. harrisoni* when exposed directly to any of the three commonly used agricultural pesticides, *ie*, carbaryl (carbamate), malathion (organophosphate) and cypermethrin (pyrethroid) (Pothikasikorn *et al*, 2007) and three commonly used public health pesticides (DDT 2 g/m², deltamethrin 0.02 g/m², and lambda-cyhalotrhin 0.03 g/m²) (Potikasikorn *et al*, 2005). Non-contact repellency to cypermethrin (Pothikasikorn *et al*, 2007), DDT and two pyrethroids (Pothikasikorn *et al*, 2005) played a significant role in the escape response in *An. minimus* s.s.

Experimental hut studies in Thailand showed that pre-spray biting activity of natural populations of *An. minimus* females peaked from 07:00-10:00 PM whereas post-treatment exposure continued to show greater landing activity during the first half of the evening (Polsomboon *et al*, 2008b). Comparatively large numbers of *An. minimus* females were collected from the unsprayed hut compared to the treated hut. There was a 71.5% reduction in the number of *An. minimus* caught in the DDT-treated hut as compared to the matched control hut, and a 42.8% reduction in the deltamethrin-treated hut compared to the matched nut. Blood-feeding showed a 71.5% decline in a hut fitted with DDT-treated panels

compared to 42.8% human-landing reduction in deltamethrin-treated panels (Polsomboon *et al*, 2008b). Based on this study, DDT exhibited significantly pronounced effects in overall reduction of biting activity than did deltamethrin.

Excito-repellency tests showed strong behavioral avoidance in the Thai deltamethrinresistant colony of *An. minimus* s.s. with more than 50% of the test population escaping from the test chambers within 30 minutes, whereas repellency was less pronounced in the susceptible *An. minimus* s.s. colony (Chareonviriyaphap *et al*, 2004). Therefore, resistant mosquitoes may not remain exposed to the insecticide for longer periods and receive a larger dose, and may not be killed readily.

1.2.5 Insecticide resistance. In Viet Nam, pyrethroid susceptible and tolerant *An. minimus* s.l. populations were found, whereas *An. minimus* s.l. from Cambodia, Lao PDR and Thailand were susceptible to DDT and pyrethroids (Somboon *et al*, 2003; Overgaard *et al*, 2005; Cui *et al*, 2012). No *kdr* mutation was found among the populations tested in the 4 GMS countries. Generally *An. minimus* was susceptible to DDT and pyrethroids in Yunnan Province, although permethrin and DDT resistance was reported in Luxi county (Cui *et al*, 2006). Only two *An. minimus* s.l. populations showed DDT tolerance, one in western Cambodia and one in northern Viet Nam. The KDT50 values for pyrethroids of most *An. minimus* s.l. population were <20 minutes (Van Bortel *et al*, 2008).

Metabolic resistance to pyrethroid was first reported in a Thailand laboratory, using resistant-selected generations of *An. minimus* species A and this was associated with increased detoxification by over-expression of monooxygenases. The oxidases are the major contributors to pyrethroid resistance (Chareonviriyaphap *et al*, 2003b). This species was collected from an area in Prae Province in 1993 that had a history of DDT spraying for malaria control since 1950, and use of chlorinated hydrocarbon pesticides for crop protection against agricultural pests and termite protection of structures. Possible cross-resistance between deltamethrin and DDT may have been promoted from previous DDT use in the area and the selection of resistance by one insecticide contributes to a much broader spectrum of resistance, including insecticides a mosquito does not normally encounter. There is a need to setup monitoring systems for the early detection of operationally unacceptable levels of resistance and conduct research on cross-resistance.

1.3 Sundaicus complex

The Sundaicus complex belongs to the Ludlowae Group of the Pyretophorus Series, *Anopheles* (*Cellia*) (Table 2; Harbach, 2004). Members of the complex are widely distributed from northeastern India through Myanmar, Thailand, Cambodia, Malaysia, southern Viet Nam, and southwards through the Nicobar and Andaman Islands, including the islands of Java,

Sumatra, Borneo and Sulawesi (Reid, 1968). The Sundaicus complex comprises *An. epiroticus* (= *sundaicus* A) and *An. sundaicus* (= *sundaicus* B, C, D and E). *Anopheles sundaicus* B (from Sumatra and Java, Indonesia), *An. sundaicus* C (from Sumatra) and *An. sundaicus* D (from India) are cytogenetically different (Sukowati *et al*, 1999; WHO SEARO, 2007) but their taxonomic positions need to be clarified. *Anopheles epiroticus* occurs in Thailand, Cambodia, Malaysia, Singapore and Viet Nam (Linton *et al*, 2005; WHO SEARO, 2007).

Anopheles sundaicus species A of Southeast Asia was formally renamed *An. epiroticus* (Linton *et al*, 2001, 2005) based on morphological characters and ribosomal DNA-internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome oxidase I (COI) sequences. After comparing populations of *An. sundacius* from Viet Nam, Thailand and Malaysian Borneo (Dusfour *et al*, 2007a), Dusfour *et al* (2007b) established three lineages by analyses of Cyt-b and COI (mtDNA) and confirmed the presence of *An. sundaicus* s.s. in Malaysian Borneo, the distribution of *An. epiroticus* from southern Viet Nam to peninsular Malaysia and recognized a distinct form in Indonesia that is named *An. sundaicus* (Dusfour *et al*, 2007a). Phylogeographic and speciation analysis suggests that these three species were separated during the Early Pleistocene (1.8-0.78 Myr) and experienced bottlenecks followed by a genetic expansion in more recent times (Dusfour *et al*, 2007). Another sibling species, *An. sundaicus* species D from Nicobar Islands, India was identified using cytogenetics (Nanda *et al*, 2004) and ITS2 sequence (Alam *et al*, 2006).

1.3.1 Bionomics. The bionomics of *An. sundaicus* s.l. in GMS countries are poorly known due to little available data. Dusfour *et al* (2004a) provided a detailed review of the bionomics of *An. sundaicus* s.l. and of the sibling species identified across the range of their distribution in the Oriental region. *Anopheles epiroticus* occurs along the continental coast from southern Viet Nam (below the 11th parallel) to Cambodia, Thailand, peninsular Malaysia and Singapore whereas *An. sundaicus* s.s. is currently only known on Malaysian Borneo.

1.3.2 Immature stages. Members of the Sundaicus complex are commonly found in the coastal areas, and their larvae and other immature stages develop primarily in habitats with salinity ranging from low and brackish to sea water concentrations. They also develop freshwater inland, particularly in northeastern India, Car Nicobar Island, peninsular Malaysia, Malaysian Borneo (Miri, Sarawak), northern Sumatra and Java, Indonesia. These species often occur in distinct areas along the coast of Thailand and Cambodia. The larvae generally require sunlit habitats with pooled stagnant water, green algae and non-invasive vegetation. They also occur in ponds, swamps, lagoons, open mangrove, rock pools, coastal shrimp and fish ponds, and irrigated inland seawater canals. In southern Viet Nam, *An. epiroticus* commonly occurs in shrimp and fish farms, with unknown economic adverse effects (Sinka

et al, 2011; WRBU, 2012). In particular larvae of *An. epiroticus* occur in large open stagnant brackish water, sunlit pools that appeared to be permanent rather than transient. In Rayong Province, Thailand, *An. epiroticus* larvae were found in cement tanks due to lack of natural breeding places. Salinity of these fresh, brackish and salt water habitats ranged from 0.5 to 119.4 g/l, which is similar to the reports of Linton *et al* (2001), Nanda *et al* (2004) and Komalamisra *et al* (2006) who found that *An. sundaicus* s.s. or *An. sundaicus* D from Malaysia, India and Thailand, respectively can breed in brackish and fresh water habitats. *Anopheles epiroticus* larvae coexisted with filamentous algae at a pH range of 8.2-8.7, similar to 7.0-8.5 pH range from studies in India, Viet Nam, Indonesia (Dusfour *et al*, 2004b), Myanmar (Oo, 2003) and Thailand (Komalamisra *et al*, 2006). Other physical factors such as dissolved oxygen (highest, 6.27 mg/l, in November; lowest, 3.46 mg/l, in March), water temperature (24.6-32.8°C) and exposure to sunlight were noted.

The maximum larval density of *An. epiroticus* was >140 larvae per dip in May, and this species co-exists with *Culex* and *Aedes* species in Rayong Province (Sumruayphol *et al*, 2010) and with *Cx. sitiens* Wiedemann and *Cx. quinquefasciatus* in the tsunami-affected areas of Phang-nga, southern Thailand (Komalamisra *et al*, 2006). In Myanmar, *An. sundaicus* is confined to the coastal areas of Rakhine State, Tanintharyi Division and the lower reaches of the Ayeyarwady Region (delta area) where the creeeks are subject to tidal influence (Oo *et al*, 2004). The main breeding habitats in Myanmar are lakes that are close to inshore areas with a mixture of saline and freshwater, shallow water in rice fields, tanks and ponds with heavy aquatic vegetation (Oo, 2003). Larvae were recorded from brackish pools in the intertidal zones near the seawater inlet on the Myanmar-Bangladesh border (Oo, 2003) and among rice fields developed for shrimp farming using brackish water in the Mekong delta in Viet Nam (Trung *et al*, 2004). *Anopheles epiroticus* and *An. dirus* s.l. overlap together from one site on the coast of Cambodia where salt water and evergreen forest merged in a 3 km buffer around the collection site (Obsomer, 2010).

1.3.3 Adult stages. *An. epiroticus* was collected resting inside human habitations and cattle sheds in Myanmar (Oo, 2003; Oo *et al*, 2004), Thailand (Sumruayphol *et al*, 2010) and Viet Nam (Linton *et al*, 2005; Trung *et al*, 2004, 2005). Females are mainly anthropophilic and exhibit both endophagic and exophagic feeding habits (Trung *et al*, 2005), although they feed indiscriminately on man or cattle in Myanmar (Oo, 2003). Generally, the biting cycle pattern increased during 06.00-08.00 PM with a peak biting activity between 08:00 PM and 03:00 AM depending on the locality; in Rayong Province, the peak was 12:00 PM with a maximum biting density of 6.6 bites/person/hour (Sumruayphol *et al*, 2010). *Anopheles epiroticus* exhibited high exophilic behavior even in control experimental huts installed in the Mekong delta of southern Viet Nam (Van Bortel *et al*, 2009), corroborating previous observations on

the behavior of this species (Trung et al, 2005).

In the Mekong delta and Rayong Province, *An. epiroticus* and *An. sundaicus* occurred at high densities, 190 bites/man/night (Trung *et al*, 2004) and 37.6 bites/person/half night (Sumruayphol *et al*, 2010), respectively. Rainfall has no effect on mosquito density in Rayong Province where *An. epiroticus* was caught throughout the year with the highest human biting rate in September, a high rainfall month (212.2 mm precipitation) and with the lowest rate in January, a dry-cool month.

The recent changes in land use from rice cultivation to shrimp farming probably explain the increase of *An. epiroticus* in Mekong delta (Trung *et al*, 2004) whereas seasonal variation in phenology account for the variation in biting densities in Rayong Province (Sumruayphol *et al*, 2010). Low survival and parous rates and the absence of sporozoite-positive *An. epiroticus* may reflect its low vectorial status that could explain the very low malaria incidence rate (1.9 case/100 persons/year) in the Mekong delta (Trung *et al*, 2004). On the other hand, the high parity rate (74%) and presence of infective mosquitoes in the dry and early rainy seasons (human *Plasmodium* sporozoite rate of 0.97% comprising six specimens positive for *P. falciparum* and three positive for *P. vivax*) resulted in an annual entomological inoculation rate (EIR) of 76.6 infective bites/person/year, or one infective bite every five day period. Infected mosquitoes largely contributed to the coastal malaria outbreaks and a total of 85 malaria cases were reported in Pak Nam village,Rayong Province during 2002 to 2008 (Sumruayphol *et al*, 2010).

In Myanmar, high densities of *An. sundaicus* are partly responsible for regular annual malaria transmission in certain areas and probably irregular local outbreaks in some localities (Oo *et al*, 2004). Sporozoite rates of 0.66% and 1.33% were reported in Rakhine State, Ayeyarwady Division and the identical bionomics of *An. sundaicus* in this State probably suggests that the same vector species is responsible for local outbreaks in other coastal areas (Oo *et al*, 2004).

1.3.4 Response to control interventions. The biting rhythm of *An. epiroticus* with a late night peak indicates the suitability of ITNs in malaria control in Viet Nam (Trung *et al*, 2005). To mitigate malaria outbreaks among the semi-immune population in South Andaman, India, high coverage and community acceptance of ITN/LLINs were very effective against *An. sundaicus* populations which proliferated due to large numbers of permanent brackish water bodies in paddy fields (Kumari *et al*, 2009). However it is not possible to attribute the effectiveness of this intervention as additional round of DDT (50% wdp) residual spraying, weekly fogging operations with malathion and larviciding were implemented in affected areas especially in relief camps and other temporary shelters during the post-tsunami period.

Experimental hut studies showed that deltamethrin conventional treated nets (CTN) and LLINs protect individuals against a pyrethroid resistant *An. epiroticus* (as defined by the WHO tube bioassay) from the Mekong region (Van Bortel *et al*, 2009), where insecticide resistance is caused by a metabolic mechanism (Verhaeghen *et al*, 2009). The personal protection ranged from 67% for deltamethrin CTN washed 5 times to 85% for unwashed PermaNet 3.0 LLIN. Pyrethroid resistance in the Mekong malaria vector did not seem to alter the well-known deterrent effect of pyrethroids despite the lack of baseline data on the deterrent effect on susceptible *An. epiroticus* population.

1.3.5 Insecticide resistance. Anopheles epiroticus is susceptible to DDT and pyrethroids in Thailand and probably in Cambodia, but showed pyrethroid resistance (eg, permethrin, deltamethrin, alpha-cypermethrin and lambda-cyhalothrin) in the Mekong region of Viet Nam (Van Bortel et al, 2009). No kdr mutation has been observed and biochemical assays suggest an esterase mediated pyrethroid detoxification (Verhaeghen et al, 2009; Cui et al, 2012). Resistance to 0.05% deltamethrin was reconfirmed in the wild population (mortality 75%) from Bac Lieu, Viet Nam in October 2008 (WHO, 2009a). Metabolic resistance is believed to induce a loss of efficacy in An. epiroticus (Van Bortel et al, 2009) but this has not reduced the protective efficacy of deltamethin CTNs and LLINs (see RESULTS section 1.3.4). The resistant status of An. epiroticus from Viet Nam clearly contrasts with the one observed in Thailand and Cambodia where this species was found to be fully susceptible based on the same discriminating dose. Anopheles epiroticus collected from the tsunami-affected area of Phang-nga Province, southern Thailand were susceptible to four diagnostic doses, ie, 5% malathion, 0.75% permethrin, 0.05% deltamethrin and 4% DDT (Komalamisra et al, 2006). The LT₅₀s of 5% malathion, 0.75% permethrin, 0.05% deltamethrin and 4% DDT were 44.7 minutes, 10.4 and 9.7 minutes, respectively.

Van Bortel *et al* (2009) pointed out that the observed pyrethroid resistant levels in Viet Nam as measured by WHO bioassays (mortality between 50-80%) might not be high enough to induce an operational impact. The expectation that PermaNet 3.0[™], designed for controlling insecticide-resistant populations will not have an additive impact in this context (Van Bortel *et al*, 2009) was confirmed by a WHOPES working group who considered that this product was not effective in controlling mosquito populations resistant to pyrethroids or to prevent the spread of pyrethroid resistance (WHO, 2009a). After washing 20 times, PermaNet 3.0[™] did not induce a significantly higher blood-feeding inhibition than PermaNet 2.0[™] washed 20 times (73% versus 68%). The significant difference in proportion of blood fed *An. epiroticus* mosquitoes between PermaNet 2.0[™] and PermaNet 3.0[™] unwashed was biologically not relevant (6.6% and 4.6%, respectively). Washed or unwashed PermaNet 3.0 did not kill more resistant *Culex* mosquitoes than PermaNet 2.0[™] and the mortality rates were low (WHO, 2009a).

1.4 Hyrcanus Group

The Hyrcanus Group which belongs to the Myzorhynchus Series, *Anopheles (Anopheles)* (Table 2), has three subgroups namely Lesteri, Nigerrimus and Sinensis, for a total of about 30 species worldwide. In the GMS, Lesteri Subgroup has five species [*An. belenrae* Rueda, *An. crawfordi* Reid, *An. lesteri, An. paraliae* Sandosham, *An. peditaeniatus* (Leicester)], Nigerrimus subgroup with three species (*An. nigerrimus* Giles, *An. nitidus* Harrison, Scanlon and Reid, *An. pursati* Laveran), and Sinensis Subgroup with eight species [*An. sinensis, An. argyropus* (Swellengrebel), *An. kunmingensis* Dong and Wang, *An. kweiyangensis* Yao and Wu, *An. liangshanensis* Kang, Tan and Cao, *An. nimpe* Nguyen, Tran and Harbach, *An. pullus* Yamada, *An. vietnamensis* (Nguyen, Tran and Nguyen)].

According to Sinka *et al* (2011), *An. sinensis* is widely distributed in the southern Asia from Afghanistan to northern China, Japan, Korea, Taiwan and southward into western Indonesia (Sumatra and West Kalimantan). Recently, Rueda *et al* (2007) provided an updated distribution of the Hyrcanus Group in China based on published records and original observations. *Anopheles sinensis* was recorded in Inner Mongolia (Cao *et al*, 2011). It is the most widely distributed species in China (found in 21 provinces and 2 cities), followed by *An.lesteri* (= *An. anthropophagus*; 15 provinces), *An. pullus* Yamada (12 provinces) and *An. kweingyangensis* Yao and Wu (11 provinces) (Rueda *et al*, 2007). *Anopheles sinensis* was the second most common species collected by UV light traps from northwestern Yunnan Province (Sun *et al*, 2009), the most dominant species in Manguo (1,400 m above sea level) and second dominant species in Manen (1,000 m asl) in Yunnan Province near the China-Myanmar-Lao PDR border (Wang *et al*, 2011).

There was some confusion when *An. anthropophagus* was described by Xu and Feng (1975) as a subspecies of *An. lesteri* from mosquitoes collected in Jiangsu Province, China, and then elevated to species status by Ma (1981). Wilkerson *et al* (2003), however, syno-nymized *An. anthropophagus* with *An. lesteri* based on identical ITS2 sequences found in *An. lesteri* from its type locality in Laguna Province, Philippines, and *An. anthropophagus* from Jiangsu Province, China. To further clarify and stabilize the taxon, Rueda *et al* (2005b) described the neotype of the *An. lesteri* from mosquitoes collected in Calauan, Laguna, Luzon, Philippines, and reference specimens are currently deposited in the National Mosquito Collections, Smithsonian Natural History Museum, Washington, DC.

Furthermore, given that *An. lesteri* is easily mistaken with *An. sinensis* and other members of the Hyrcanus Group, previous published reports of its occurrence, distribution and bionomics may be inaccurate, particularly in Japan, Korea and China. Molecular analysis of nucleotide sequence alignment of ITS2 regions showed that *An. lesteri* from Japan and *An. lesteri* (as *An. anthropophagus*) from China are the same species (Hwang *et al*, 2006).

Ribosomal DNA PCR assays were used to identify *An. sinensis* in Yongcheng city (Liu *et al*, 2011, 2012). Using diagnostic RFLP-PCR, specimens identified morphologically as "*An. anthropophagus*" in the adult and egg stage from one location in Quangdoing Province were found to be *An. sinensis*, while specimens from Liaoning Province which were variable in their egg morphology, were identified as *An. lesteri* (= *An. anthropophagus*; Gao *et al*, 2004). The presence of *An. lesteri* (= *An. anthropohagus*) in this province extends the range of this species north to 42°N.

Intraspecific sequence divergence was slightly greater than the 2% threshold within *An. sinensis.* The first barcodes of the cytochrome c oxidase subunit 1, the CO1 gene, for mosquitoes (morphotypes of 15 genera and 122 species and subspecies) in China provides further evidence of the effectiveness of DNA barcoding in identifying specimens collected from the field as part of disease surveillance programs (Wang *et al*, 2012a). Recently, Walter Reed Biosystematics Units has generated CO1 barcode sequences (658 bp) for about 421 species of 458 recognized *Anopheles* species worldwide, including members of the Hyrcanus Groups and other vectors groups in the GMS. CO1 sequence data, however, are only publicly available for about 89 *Anopheles* species. Through concerted efforts, barcoding of the remaining species must be completed to provide quality identifications and positive impact on accurate vector incrimination and vector control.

In the deforested areas of north central Viet Nam, *An. sinensis* played a relatively major role in malaria transmission (Sanh *et al*, 2008) compared to Lao PDR and Thailand where they were reported as possible vectors (Rosenberg *et al*, 1990; Rattanarithikul *et al*, 1996). Large numbers of *An. sinensis* were recorded in Khammouane Province, Lao PDR (on the border with Quang Binh Province, Viet Nam) where 44.2% and 21.4% of mosquitoes were taken from animal bait collections and HLC, respectively (Toma *et al*, 2002). Although *An. sinensis* is associated with rice growing in the GMS, the reasons for low densities recorded in surveys in Thailand and Lao PDR are unknown.

1.4.1 Immature stages. Of 60 breeding sites of *An. sinensis* surveyed in Yongcheng City, larvae were found in 8 (13.3%) river fringes, 26 (43.3%) ponds, 23 (38.3%) puddles, and 3 (5.0%) irrigation/drainage ditches (Liu *et al*, 2012). About 94.9% of *An. sinensis* larvae inhabited relatively large and medium-sized water bodies, with depths between 0.5 m and 1.0 m, chemical oxygen demand (COD) lower than 2 mg/l (75%), <0.4 mg/l ammonia nitrogen (86.7%) and <150 mg/l sulfate (58.3%) concentrations. Effective larval control should target breeding site type, water depth, COD, ammonia, nitrogen and sulfate in malaria endemic areas where *An. sinensis* is the primary vector. The recent WHO Interim position paper on larviciding in Sub-Saharan Africa noted that "*in general larviciding should be considered for malaria control (with or without interventions) only in areas where the breeding sites are few,*

fixed and findable" (WHO, 2012b). As large tracts of arable land are used for rice fields and farming, the IWDI and WDCR methods (see RESULTS section 4) could be incorporated into larval source management with emphasis on the benefits to rice productivity and long-term mosquito control.

1.4.2 Bionomics and malaria outbreaks. Although the vectorial capacity of *An. sinensis* is much lower than *An. lesteri* (= *An. anthropophagus*), large populations are responsible for maintaining a low endemicity in the plains of China. It is an important vector in the temperate and sub-temperate countries north of 34°N, *ie*, Japan, Democratic Republic of Korea and Republic of Korea but its role in the tropics is negligible.

The vectorial efficiency of *An. sinensis* was increased during the hottest periods when people frequently slept outdoor near their fields and unprotected by bed nets (Liu *et al*, 2004). Local outbreaks of malaria can be sustained by *An. sinensis* during such periods, especially if the other entomological parameters favoring transmission are also present, *eg*, a high sporozoite rate and an aging mosquito population. Recent outbreaks of malaria and re-emergence were associated with the predominant vector *An. sinensis* in the Huanghuai valley of central China (including the four provinces of Anhui, Henan, Hubei and Jiangsu) (Zhou *et al*, 2010; Pan *et al*, 2012) which also plays an important role in the maintenance of *P. vivax* malaria transmission (Liu *et al*, 2011). It was considered to be the sole potential vector of *P. vivax* malaria in Yongcheng city with a 2.78-fold vectorial capacity in 2010 (0.4983) than in the 1990s (Zhou *et al*, 2010; Liu *et al*, 2012).

In animal choice trap studies, pigs, goats and calves were more attractive to *An. sinensis* than dogs, humans and chickens (Liu *et al*, 2011). As host seeking activity mainly occurred from 07:00 PM to 09:00 PM, it was proposed that vector control strategy should target the pre-bedtime interface of human activity with domestic animals (Liu *et al*, 2011). The HBI of fed *An. sinensis* including mixed meals was 2.94% and 3.70% in Yiongcheng City (Liu *et al*, 2011) and 6.42% and 6.67% in Huaiyuan and Yongcheng counties (Pan *et al*, 2012), respectively. Increasing use of agricultural machinery and the reduction of farm cattle were responsible for the increased HBIs which were 12 times higher than in the 1990s when the number of pigs and farm animals were relatively abundant (Pan *et al*, 2012). The corresponding expectation of life and vectorial capacity of *An. sinensis* were 0.36-0.47 and 0.55-0.77, respectively, in Huanhuai valley of central China where the outbreaks occurred, and these figures were much higher than in the malaria-free years (Pan *et al*, 2012). Vivax malaria outbreaks in this setting is highly related to enhanced transmission ability and increased densities of *An. sinensis*, the agricultural habits of local residents and the scarcity of farm livestock which served as zooprophylaxis.

In a GIS analysis of 357 malaria cases and 603 water bodies in Huang-Hai river of central China, 74% of malaria cases were distributed around the 60.9 m median distance from the positive households to the nearest breeding sites (Zhou *et al*, 2010). That 94.2% of malaria cases occurred in areas with *An. sinensis* suggests that the vectorial capacity has increased in relation to *An. lesteri* (= *An. anthropophagus*) which played a key role in severe malaria epidemics in the last century (Zhou *et al*, 2010). The vectorial capacities of *An. sinensis* in the two sites (Huaiyuan and Yongcheng counties) were 0.69 and 0.47, respectively which were 4.12 and 2.78 times higher compared to that of 1990s (0.1686).

In view of the rapid, extensive economic development involving environmental change in the southern province of Guangdong, China, a three-year survey was conducted between 2004 and 2006, to update the obsolete mosquito fauna records. Light traps in non- or sparsely populated areas caught 5,995 mosquitoes, the most common species being *An. sinensis, An. maculatus, An. minimus* and other culicine genera (Jin and Li, 2008). Malaria vectors in this endemic province included *An. sinensis, An. lesteri* (= *An. anthropophagus*), *An. minimus* and *An. jeyporiensis*.

The ubiquity of DDT resistant in *An. sinensis* is striking as 89% of 27 regions in five provinces (Huebi, Sichuan, Fujian, Anhui and Yunnan) reported this problem (Cui *et al*, 2006). However, this species and *An. lesteri* (= *An. anthropophagus*) showed susceptibility and moderate resistance to pyrethroids.

Secondary and infective malaria vectors such as *An. pseudowillmori* (Theobald) were confirmed by Multiplex PCR and nested PCR from cow-baited and human-baited net traps and CDC light traps in Motou county, Linzhi Prefecture located in the south-eastern part of the Tibet Autonomous Region (Wu *et al*, 2009). Mosquito surveys conducted in Minhe county, Qinghai-Tibet plateau revealed the first record of *An. nigerrimus* in the far north of China (Li *et al*, 2010).

Anopheles belenrae adult females that were collected while resting in cow sheds and from light traps were found positive for *P. vivax* in South Korea (Rueda *et al*, 2010). This species has also been collected in China (Rueda *et al*, 2007), but its abundance, distribution and vectorial capacity need to be studied. Similar to other Hyrcanus Group species (*eg, An. kunmingensis, An. kweiyangensis, An. liangshanensis, An. pullus*), the distribution of *An. belenrae* in the GMS (aside from China) is still unknown.

1.5 Other Anopheles vector groups

1.5.1 Maculatus Group. The Maculatus Group belongs to the Neocellia Series, *Anopheles (Cellia)* (Table 2). This group includes three subgroups for a total of eight species, of which six species are found in the GMS, including Maculatus subgroup which comprises *An. dra*-

vidicus Christophers (= Form C) and *An. maculatus* Theobald s.s. (= B); Sawadwongporni Subgroup with *An. notanandai* Rattanarithikul and Green (= G) and *An. sawadwongporni* Rattanarithikul and Green (= A), as well as unassociated subgroup species with *An. pseudowillmori* (Theobald) (= I), and *An. willmori* (James) (= H). Non-GMS species such as *An. greeni* Rattanarithikul and Harbach (= D) and *An. dispar* Rattanarithikul and Harbach (= J) occur in the Philippines. Chromosomal forms K maybe another species belonging to the group (Walton *et al*, 2007), while forms B and E from Peninsular Malaysia are probably similar to *An. maculatus* s.s. in Thailand (Rongnoparut *et al*, 1999). The status of forms E and K from Thailand in terms of whether they are conspecific or undescribed species needs to be determined. *Anopheles maculatus* s.s. and *An. sawadwongporni* are widely distributed in Thailand, except for the far south, whereas *An. maculatus* E is found throughout the peninsular region (Rattanarithikul *et al*, 2006).

Members of this Group are found in or near hilly areas and mountainous areas. Typical larval habitats are ponds, lakes, swamps, ditches, pits wells, pools (grassy, sand, ground, flood, stream), stream margins, seepage springs, rice fields, animal foot prints, wheel tracks, artificial containers, holes in fallen trees and bamboo stumps. Larvae of this Group are usually found in recently cleared areas with disturbed soil. *Anopheles willmori* occurs at altitudes between 990-1,475 m in northern Thailand, with larvae found only in stream margins. *Anopheles pseudowillmori* is usually found in rice fields, stream margins, ponds, pits and well. Larvae of these species occur in sand pools along the Mekong River in northeastern Thailand (Rattanarithikul *et al*, 2006; WRBU, 2012). Larvae of *An. sawadwongporni* are common in stream margins, stream pools, flood pools, swamps, rice fields, rock pools, ground pools, and animal foot prints, particularly in Tak Province, Thailand (Jaichapor *et al*, 2005).

Anopheles maculatus is considered as a primary vector of malarial parasites and vector of filarial worm, *Wuchereria bancrofti.* Anopheles pseudowillmori is a secondary malaria vector in northwestern Thailand along the Myanmar border (Green *et al*, 1991). It is also the predominant malaria vector in the Motuo County, Tibet (Song *et al*, 2009). Anopheles willmori and An. sawadwongporni are also confirmed vectors in Thailand, with the latter species having sporozoites rates between 1% and 2% (Rattanarithikul *et al*, 1996). Natural infections of Pv phenotypes were observed in An. maculatus (one positive each for VK210 and VK247) and An. sawadwongporni (one positive each for VK247 and Pf) (Coleman *et al*, 2002), suggesting the potential role of this vector in habitats undergoing land use and environmental changes in Thailand.

There is a need to revise the taxonomy of the Maculatus Group because many populations exhibit too many variations in their ecology, behavior and vectorial capacity within their geographical distributions, compared with the typical *An. maculatus* s.s. In association with *An. minimus* s.l. (see RESULTS section 1.2.4), the density of *An. maculatus* s.l. significantly increased in the wet season from the period 1997-1989 to 1990-1999 in Mae Hong Son Province, Thailand (Suwonkerd *et al*, 2004). Increasing vector populations were a contributing factor to the consistent high malaria incidence in this province, and this supports the entomological observations that *An. maculatus* complex play a larger role in malaria transmission in northern Thailand than previously assumed (Suwonkerd *et al*, 2004).

1.5.2 Subpictus Group. The Subpictus Group belongs to the Pyretophorus Series, *Anopheles (Cellia)* (Table 2). It includes *An. indefinitus* (Ludlow), *An. subpictus* Grassi and *An. vagus* Doenitz. *Anopheles subpictus* s.l. has four forms (A, B, C and D) that occur in Southeast Asia: form A in Indonesia and the Philippines, form B in Thailand, Indonesia and the Philippines, and forms C and D in Thailand (Baimai *et al*, 1996). In other Asian countries, the specific distribution of these four forms is not known and taxonomic studies are needed.

Members of the Subpictus Group are typically found in freshwater habitats. *Anopheles vagus* Doenitz is the most abundant species of the Group, with larvae and pupae usually found in numerous ground water habitats including rice fields, swamps, marshes, bogs, ditches, pits, wells, grassy pools, flood and stream pools, stream margins, wheel tracks, tire depressions, animal footprints, rock pools, rock holes, crab holes, and artificial containers. Larvae of *An. indefinitus* (Ludlow) are commonly found in rice fields, ponds, grassy pools, seepage pools and stream margins. Larvae of *An. subpictus* s.l. usually occur in rice fields, ditches, wells, ground pools, animal foot prints, and artificial containers (Rattanarithikul *et al*, 2006; WRBU, 2012).

1.5.3 Barbirostris Group. The Barbirostris Group belongs to the Myzorhynchus Series, *Anopheles (Anopheles)* (Table 2). The Barbirostris subgroup of this group in the GMS includes five species (*An. barbirostris* Van der Wulp s.l., *An. campestris* Reid, *An. donaldi* Reid, *An. hodgkini* Reid, *An. pollicaris* Reid). *Anopheles barbirostris* s.l. includes 3 genetic forms, A, B and C, with type locality in Indonesia. In the GMS, the sibling species composition of *An. barbirostris* s.l. is not known. *Anopheles barbirostris* and *An. campestris* are the most common species of this group, and they are closely associated with humans. Their immature stages are usually found in rice fields, stagnant ditches between rows of coconut palms, earthen wells, and other ground water habitats. The other three species usually occur in forest habitats, including shaded pools, ground pools and rock pools. *Anopheles campestris* and *An. donaldi* are considered vectors of malarial parasites in Thailand, and both malarial parasites and filarial worms in other non-GMS countries (Rattanarithikul *et al*, 2006; WRBU, 2012). Two specimens of *An. campestris* were naturally infected with VK210 sporozoites and one *An. hodgkini* specimen positive for VK247 (Coleman *et al*, 2002), suggesting the potential roles of secondary vectors in habitats experiencing land-use and environmental

changes in Thailand. *Anpheles barbirostris* was for the first time confirmed as a vector of *P. malariae* in Cambodia (Durnez *et al*, 2011).

2. Use of insecticides for vector control

Vector control is an important element of strategies used to control malaria and other major vector-borne diseases. In recent years, interventions using insecticides have been scaled up in many countries. Effective systems for pesticide management require procedures to ensure judicious use of insecticides, manage insecticide resistance, and reduce risks to human health and the environment. WHO (2011b) released a report on the actual use of insecticides covering 10 years (2000-2011) by vector control programs in WHO's Member States, including GMS countries. The purpose of this survey was to inform decisions about the use of insecticide to control vector-borne diseases; for information exchange and regional collaboration; and as basis for managing chemicals.

The data also show a high use (in terms of tonnes of active ingredient) of organochlorines and organophosphates for malaria control in the Southeast Asia Region but a decline in the Western Pacific Region. Use of pyrethroids in public health is high in both regions, especially given their low application rate and thus, their spray coverage achieved. The widespread and predominant use of pyrethroids for malaria vector control is worrisome as it exerts a high selection pressure for the development of resistance in vector populations. This concern is relevant to public health because a major tool in malaria control is the use of LLINs which depends solely on the action of pyrethroids. Hence it is critical that the susceptibility of malaria vectors to pyrethroids is preserved.

To prevent resistance from emerging at new sites, and to maintain the effectiveness of vector control interventions in the short-, medium- and long-term, the GPIRM recommends that pyrethroids should not be used for indoor residual spraying where there is high coverage with treated nets (WHO 2011b, 2012). GPIRM includes five major activities (pillars) spanning the short, medium and long term. These include: i) the planning and implementation of insecticide resistance management; ii) ensuring proper timely entomological and resistance management and effective data management; iii) developing innovative vector control tools; iv) filling the gaps in knowledge on mechanisms of resistance and impact; and v) ensuring that enabling mechanisms (advocacy, human and financial resources) are in place (WHO, 2012).

2.1 Sound management of pesticides of public health importance

GMS is facing a significant burden of vector-borne diseases, such as malaria, dengue, chikungunya, arboviral diseases and scrub typhus. Over 90 million people in the Mekong Region are at risk of malaria, and dengue fever is a serious and increasing threat, with 1.8 billion people at risk in the Asia-Pacific Region. Interventions to effectively control vectors

for these diseases depend largely on the use of pesticides. The burden to public health caused by nuisance pests (mostly insects and rodents) is also significant in the GMS, leading to the use of considerable volumes of pesticides for domestic pest control, agriculture, and private sector. However, comprehensive statistics on pesticides used for such purposes are not widely available in the GMS. The amount of pesticides consumed in Thailand was reported to be 39,904 metric tons of active ingredient (WHO, 2006a).

A recent questionnaire study on pesticide use patterns indicated that pesticides are available and widely used in crop production in rural Phitsanulok, Thailand (Plianbangchang et al, 2009). This includes the use of endosulfan which has been banned by the Thai government since 2004 for health and environmental reasons. Inappropriate pesticide use was common among small-scale farmers; for example, they did not wear suitable personal protection, apply pesticides correctly, or discard the waste safely. Farmers frequently depend on commercial advertisements for the best pesticide to use. Findings from this survey clearly suggest the necessity to reduce possible health and environmental risks associated with pesticide use through public policies encouraging farmers to change their pest management methods from chemical based to methods that are safer and more environmentally friendly. Educational interventions are also essential for promoting safety during all phases of pesticide handling which is consistent with WHO (2011d) advice for Member States and public health programs. A review of a health promotion program on safe use of pesticide conducted for a group of Thai farmers in Ratchaburi Province showed that the mean scores of KAP in the posttest were significantly higher than the pretest, indicative of an effective training program (Janhong et al, 2005). Results from this study provided health professionals with information to develop more effective prevention and intervention programs, such as health improvement, change traditional methods of pesticide utilization and provide a self-help program of first aid and basic CPR for poisoning cases.

A case-control study assessing the effects of agricultural pesticide use on semen characteristics among rice farmers of Kienxuong District, Thaibinh Province, Viet Nam showed that male farmers without personal protective equipment (PPE) who had not received pesticide use training were at greater risk of having abnormal semen compared to controls (Tuc *et al*, 2007). This finding should contribute significantly to pesticide regulation especially the requirement of PPE for farmers and is consistent with WHO (2011d) advice for public health workers.

Easy accessibility of pesticides is also a reality in many developing countries such as China (Liu *et al*, 1997). These pointed out the lack of effective control regulations and safe strategies, a similar conclusion from a 2010 global survey (WHO, 2011d). A recent study focusing exclusively on pesticide regulation aimed at reducing occupational exposures

among Vietnamese farmers identified several measures that would help to improve Viet Nam's pesticide regulations (Phung *et al*, 2012). These include enhancing pesticide legislation, clarifying the specific roles and active involvement of both the environmental and health sectors; performing a comprehensive risk-benefit evaluation of pesticide registration and management practices; improving regulations on pesticide suspension and cancellation, transport, storage and disposal; developing import and export policies and enhancing pesticide-related occupational safety programs. These measures are consistent with the WHO Regional framework for action on the sound management of public health pesticides in the Western Pacific, 2012-2016 (WHO WPRO, 2011). As regulations alone may not be sufficient to reduce the risk posed by pesticides, the study recommended more comprehensive needs assessments involving the farming community (Phung *et al*, 2012).

Similarities in the landscape of public health pesticide registration and management practices among the agricultural and public health sectors are quite common in the GMS. A recent global study which included GMS concluded that national capacity to manage public health pesticides throughout their life-cycle was inadequate in most of the countries (WHO, 2011c). Issues noted in the 2010 WHO survey among Member States in the Region were: inadequate regulations of public health pesticides; the lack or incomprehensiveness of pesticide legislation; inadequate coordination in registration of public health pesticides (PHPs); the lack of published guidelines for registration of PHPs; the lack or inadequate regulations; the generally inadequate compliance and enforcement of regulations; the presence of substandard, illegal and counterfeit PHPs on the market; the low capacity of Member States for PHP quality control and their alarmingly low capacity for disposal of pesticide containers and pesticide-related waste, as critical issues requiring urgent action; and called for sound management of PHPs. The challenge was further compounded where adequate capacity for quality control and enforcement of pesticide regulations and for monitoring of applicator exposure has not been established under decentralized health systems.

3. Impact of land-use changes

Vanwambeke *et al* (2007a,b) provided four scenarios at the village level to account for the diversity in environmental and social contexts, such as land-cover change, and in combination with human behavioral changes or changes in the density of mosquito habitats. The working hypothesis was that changes in land use, preventive measures and control policies will not necessarily have the same effects in different villages. These changes can influence disease transmission and mosquito populations, and can result in an increase or a decrease of the risk. In northern Thailand, the varied and heterogeneous landscape is characterized by natural forests and valleys. The former is dominated by dry dipterocarp forests and, on moister sites, mixed deciduous forests, whereas the fertile valley bottoms comprise of cultivated and irrigated plots which include a dry-season crop other than rice and upland plots of field or tree crops (Vanwambeke *et al*, 2007b).

In the first scenario, a 50% decrease in forest cover in the area within flight distance from the villages resulted in a change of *An. minimus* s.l. and *An. maculatus* populations in the forested site and for the valley site. For example, "*in the forested site, the difference in the An. minimus group was predicted to be slightly smaller than the decrease of An. maculatus group, and was proportional to the decrease in forest cover. In the valley site, the population of the <i>An. minimus group. This difference was due to the distinct distribution of habitats in the two villages: in the forested site, the village area provides approximately 4% of the <i>An. minimus group, whereas the in the less forested site, the village area provides approximately 27% of the population*" (Vanwambeke *et al,* 2007b).

In the second scenario, orchard expansion either takes place following deforestation at a certain distance from villages or by conversion of existing fields near villages. Decreases of An. dirus were observed following deforestation and the subsequent introduction of rice cultivation and cassava and sugar plantations, and increased only when rubber plantations were introduced to the area (Yasuoka and Levins, 2007). Similarly the decrease of An. minimus populations was associated with deforestation and an increase in all subsequent agricultural activities, eg, rice cultivation and cassava, sugarcane, and coffee plantations (Yasuoka and Levins, 2007). In a non-transmission area in Chiang Mai Province, northern Thailand, An. minimus s.l. density decreased between 1977 and 1999 (Suwonkerd et al, 2004) and this was associated with increased landscape diversity and forest fragmentation (Suwonkerd et al, 2002). Landscape diversity was characterized by 12% and 6% increase in fruit orchard and agricultural area acreage from 1983 to 1995, respectively. Forest fragmentation was characterized by 8% reduction of forest cover from 1983 to 1995 (Suwonkerd et al, 2002). However, in transmission areas in the same province where forest cover was extensive (characterized by 4% reduction from 1983 to 1995) and landscape diversity was low (characterized by 3% increase in grass vegatation/growth, and 2% increase in agriculture from 1983 to 1995), malaria vector densities increased during the same period (Suwonkerd et al, 2002, 2004). In a comparable area in northern Thailand, Overgaard et al (2003) found that Anopheles diversity and density were generally higher in forested areas with low landscape diversity than in agricultural areas with high landscape diversity. Increased landscape diversity probably creates negative secondary effects on vector density or unattractive conditions for An. minimus breeding due to changes in stream corridors, increased water pollution and forest fragmentation. In northern Thailand, Vanwambeke et al (2007a) showed that turbid and temporary water, and the presence of algae and excrement decreased the probability of finding *An. minimus* s.l. larvae. Similar effects were observed in northern Viet Nam where recent agricultural development linked to modified irrigation might have disturbed mosquito habitats causing low density of *An. minimus* s.l. (only 3 specimens) (Garros *et al*, 2008). Human economic activities often increase landscape heterogeneity such as the application of pesticides in the dry season which may result in a reduction of anopheline species diversity or density (Overgaard *et al*, 2003).

Sites located in the most diverse landscapes, as indicated by the modified Simpson index >1.2, had a fivefold higher chance of harboring *An. minimus* larvae than those with the least diverse landscape, but the association with malaria transmission is inconclusive (Vanwambeke *et al*, 2007a).

As An. minimus also breeds in villages where it is in closer contact with humans, changes in housing infrastructure could potentially increase biting rate (see RESULTS section 4.2; Vanwambeke et al, 2007a). Some shift in malaria incidence from the forested areas toward fruit orchards and rubber plantations were recently noted in Cambodia, particularly reports of asymptomatic infections in sparsely forested areas (Networks, 2012). In the third scenario, other factors such as human movement and behavior and socioeconomic conditions probably play a role in the malaria transmission. Commercialization of fruit crops and orchard cultivation generally increased household income and is associated with social changes related to engagement in a market economy (Vanwambeke et al, 2007a). Farm owners in Cambodia saw the advantage of bednets being used by their workers and some would purchase nets to loan to workers, whilst others said they would be prepared to advance labourers' wages to enable workers to buy bednets from the local markets at the beginning of their stay (Networks, 2012). Non-governmental organizations such as University Research Co. and Family Health International (FHI) have been involved in the employer 'Loan Schemes' or 'Lending Schemes' to manage the LLIN loan schemes for farm owners and seasonal agricultural laborers in western Cambodia (Networks, 2012; Sean Hewitt, personal communication). FHI considered this to be an efficient and replicable scheme as it provides ready access of LLINs for migrants and remote farmers. A new retail market has recently opened up in Pailin-Phant Rolim in Cambodia which supplies nets and hammocks for mobile migrants and farm workers from the Cambodian-Thai border (Networks, 2012). Rubber tappers ingeniously adapted protective tools such as burning mosquito coils attached to head caps when they are tapping trees coinciding with An. dirus biting times (Networks, 2012; Michael Macdonald and David Sintasath, personal communication). There is a need for more studies to assess the effects and impact of agricultural intensification and orchard expansion on household integration into a market economy, awareness about disease risk and investment in preventive measures.

In the fourth scenario, construction of hydroelectric dams and downstream effects create favorable habitats for mosquitoes, often under tree cover, especially favoring *Anopheles* species that inhabit forests. Assuming a year-round 10% increase in stream habitats in forest areas, the model predicted a proportion smaller than 10% of baseline larval densities of both *An. minimus* and *An. maculatus*, with a minor seasonal effect (Vanwambeke *et al*, 2007a). This effect was related to the respective contributions of forest and village areas in the total mosquito populations in the dry and wet seasons.

Vanwambeke *et al* (2007b) concluded that land-use change impacted on mosquito populations and disease transmission risk, but its exact effect cannot be easily predicted without local-scale contextual information.

4. Environmental management

A sound knowledge of local ecosystems and the position of vector species in each ecosystem is an essential pre-requisite for implementing appropriate environmental management (Table 4) as the objective is to reduce the environmental receptivity to vector breeding and disease transmission.

Ten of 25 environmental modification studies reviewed by Keiser *et al* (2005) were from four Southeast Asian countries (China, Hong Kong, Indonesia, Malaysia, Singapore) which shared similar ecological and sociocultural features with the GMS countries. These studies were conducted in different eco-epidemiological settings (coastal urban, rural and irrigation malaria) involving *An. aconitus, An. balabacensis, An. maculatus, An. sinensis, An. sundaicus,* and different levels of endemicities and were launched before the Global Malaria Eradication Campaign, which was implemented between 1955 and 1979.

The one environmental manipulation study that was initiated after 1994 to present was intermittent irrigation of rice fields in Sichuan Province, China (Liu *et al*, 2004; Keiser *et al*, 2005). This was based on the wet-irrigation method described by Lu (1988) and over the last three decades the extended irrigation system has ensured water security and increased the area of arable land that could be farmed by intermittent wet dry/irrigation (IWDI). Additionally, rice fields that had been left flooded but fallow throughout the winter are managed by an annual cycle of wet crop/dry crop rotation (WDCR) to maximize productivity (Liu *et al*, 2004). Vector densities in houses and cow sheds were consistently higher in areas without WDCR compared to areas with WDCR (70% and 75% difference for *An. sinensis*, respectively; 64% and 81% difference for *An. anthropophagus*, respectively) (Liu *et al*, 2004). The impact of WDCR over ITN was the gradual drop of breeding sites which in turn reduced vector populations below the level required to sustain malaria transmission. However, after the suspension of ITNs in Daxing Township, Pujang County, malaria cases increased from

Table 4

Types of environmental management for controlling major vectors of malaria in the GMS (modified from Lindsay *et al*, 2004).

Major vector	Type of breeding habitat	Potential environmental intervention
An. dirus	Forest mosquito breeding in small ground pools or slow moving streams	Exposure to sun, filling, resting of new plantations
An. maculatus	Hill streams and ponds open to sunlight	Flushing (automatic siphons), shading
An. minimus	Stream margins, gravel pits and small puddles in the shade	Flushing, exposure to the sun
An. sinensis	Rice fields, irrigation ditches, large grassy pools	Flushing, intermittent wet/dry irrigation, wet crop/dry crop rotation
An. sundaicus	Partially cleared mangroves and coastal wetlands. Sagnant brackish water	Shading breeding places, weed clearing, drainage, highly salt water

3 in 1989 to 19 in 1991, and mean mosquito density rose from 0.2 to 7.9 mosquitoes per net per night. Apart from IRS/ITN and treatment of malaria cases, it appears that malaria elimination in some areas was attributed to the elimination of a critical proportion of breeding sites through IWDI and WDCR (Liu *et al*, 2004).

Some prominent environmental modifications managed by these countries reviewed by Keiser *et al* (2005) include: drainage, clearing of jungles, straightening of streams (Hong Kong), drainage of swamps, filling of small water bodies (Klang and Port Swettenham, Malaysia), drainage, tree clearing, improved house siting (coastal and hilly rubber estates, Malaysia), drainage of swamps, filling, subsoil pipes (Singapore) and drainage after rice harvest, improvement of drainage system (Cihea, Indonesian archipelago). Rubber plantations provide dense shade and potentially suitable habitats for various *Anopheles* vectors. Planning restrictions to ensure that new rubber plantations are sited well away from human settlements should help to minimize the development of new malaria transmission foci (Sean Hewitt, personal communication). Common strategies for coastal areas occupied by *An. sundaicus* in Indonesian archipelago are as follows: filling, raising of surface of seaside area, replace open drains with closed drains, drainage of fishponds within 3 km from human settlements, ban on cutting mangrove within 2 km of villages, construction of piers and filling of swamps (Yasuoka and Levins, 2007).

It is noteworthy that two countries on track with malaria elimination, Sri Lanka and Malaysia, had a long history of environmental manipulation tools used in the pre-DDT era

such as automatic siphons (Singh and Tham, 1988; WHO, unpublished report). Installed by the two governments in the 1930s, these shiphons were used to flush streams and rivers to eliminate the breeding of mosquito larvae (Worth and Subrahmanyam, 1940). Flushing creates strong currents forcing the removal of larvae from their natural breeding sites and their final stranding when the water level falls. Stream flushing also inhibited plant growth, thereby reducing opportunities for larval breeding (Singh and Tham, 1988). A cheap siphon can discharge between 0.3 to 0.6 m³/s), generating a water velocity of 0.2-0.5 m/s, and was reported to produce very successful results (Konradsen *et al*, 2004; WHO, unpublished report).

Learning from the successes and failures from the pre- insecticide era is necessary to guide future research and operational agendas focusing on environmental-control interventions (Konradsen *et al*, 2004). From the IVM perspective, interventions piloted and implemented early in the last century are still relevant today. They require co-ordination and collaboration mechanisms between different public sectors, subsidiarity and partnership. They also add resilience to the results of individual control programs and reduce costs, which is particularly important during times of global financial crisis or social unrest (Lindsay *et al*, 2004; WHO, 2004).

Some of the pioneering approaches adopted in the early 20th century to support implementation may not be acceptable or applicable today, from a social or environmental point of view. Konradsen et al (2004) cautioned that many of the permanent modifications of the environment such as jungle/forest clearing and drainage resulted in massive destruction of what today is classified as conservation ecosystems, such as wetlands, freshwater streams or mangrove swamps. As with most of the development activities during the 20th century, the implications for the natural environment were not given much attention. Similarly, some of the interventions that resulted in the forced relocations of population groups may not be acceptable by current social standards (Konradsen et al, 2004). Emergency relocation of refugees from endemic areas could provide a large reservoir of infection and, if aircraft, trains and/or buses are used, will increase the possibility of introducing exotic vectors into malaria-free areas. Drug resistant parasites will add to the difficulties of treatment (WHO, 2004). Whilst there was little evidence of any health impact assessment regarding enforced relocation of households in an irrigation project in rural Cambodia, the influx of construction workers and relocation of people affected by inundation in the Xiaolangdi multipurpose dam project, Yellow River, China was a major concern (Lindsay et al, 2004). An environmental impact assessment in 1997 identified that changes in the hydrology of the region could increase malaria in the area (Lindsay et al, 2004).

Anecdotal evidence from northeast Cambodia suggests that until recent improvements in access to healthcare, a vicious-cycle of malaria related to periodic environmental changes

existed among some ethnic minority groups (Sean Hewitt, personal communication). Traditionally swidden farming Jarai and Tampoun communities relocated their main village periodically to avoid evil spirits whenever disease and mortality reached intolerable levels. The new settlements were generally established within a few hundred meters of the original village. Trees were cleared, timber, bamboo and thatch stilt houses were constructed and fruit trees (including coconut and mango) were planted within the confines of the new settlement. Immediately after the move the health of the villagers would improve. However, as the trees grew they provided increasing levels of shade and humidity increasing the risk of various communicable diseases including malaria. When morbidity and mortality reached intolerable levels the communities would relocate again.

During informal discussions community leaders indicated that relocation occurred infrequently, once or twice in their lifetimes, resulting in a definite improvement of people's health. Surveys conducted during 2001 (Sochantha *et al*, 2006) corroborated these perceptions revealing that prevalence of malaria was generally lower in newly established shadeless settlements than in long established shaded settlements.

4.1 Tsunami, flooding and aquaculture

Global warming will raise sea levels and cause an expansion of brackish and saline water bodies in coastal areas, resulting in increased densities of salinity-tolerant vector mosquitoes and the adaptation of freshwater vectors to breed in brackish and saline waters (Ramasamy and Surendan, 2011). The initial effects of tsunami are seen in affected areas with salt water making most stagnant water bodies unsuitable for malaria vectors. However the onset of monsoon rainfall desalinates sea water making breeding sites increasingly suitable for vectors of malaria and other diseases (WHO, 2005). Following the Asian tsunami of December 2004, *An. sundaicus* s.l. increased in density in the Andaman and Nicobar islands, following the intrusion of sea water inland, concomitant with a rise in *P. falciparum* infections (Krishnamoorthy *et al*, 2005). Increased densities of *Cx. sitiens*, an established vector of arboviruses and *An. sundaicus* s.l. were also observed in an area of Thailand affected by the tsunami (Komalamisra *et al*, 2006), (Table 7). However, there was no impact on malaria incidence (Wiwanitkit, 2007) despite increases of densities of *An. epiroticus* in Thailand (Sumruayphol *et al*, 2010).

Schapira and Boutsika (2012) considered that coastal malaria has generally lost its importance in Asia due to the inefficient *An. sundaicus/epiroticus* mosquitoes which are largely endophilic and are readily controlled by IRS and ITNs. However, high densities of *An. epiroticus*, the presence of an infectious human reservoir and ideal ecological conditions were responsible for coastal malaria outbreaks in Thailand (see RESULTS section 1.3.3; Sumruayphol *et al*, 2010). Furthermore, the economic development has facilitated

good access to curative care and contributed to control of coastal malaria. Given the low disease burden except in situations of natural disasters, environmental management is not a cost-effective investment and may not be attractive to the malaria programs. The traditional environmental modification measures such as automatic sluice gates and earth embankments that proved to be effective during the pre-DDT era, did not provide immediate solutions as the physical infrastructure were destroyed following the tsunami (Krishnamoor-thy *et al*, 2005). In emergency situations with a high risk of coastal vector abundance and paucity of cattle causing a threat of malaria outbreaks in densely populated camps, rapid deployment of IRS and mass distribution of LLIN/ITNs are effective strategies. Other control measures such as the promotion of the use of ITPS (see RESULTS section 1.1.5 g) and repellents may be appropriate when IRS infrastructure and human resources are scarce or unavailable. Surveillance and prompt treatment of malaria cases should also be intensified.

Aquaculture is an expanding economic activity along tropical Asian coasts. The impact of large-scale shrimp farming resulted in local increase in the density of *An. sundaicus* s.l. (now *An. epiroticus*) in the Mekong delta of Viet Nam (Erhart *et al*, 2004; Trung *et al*, 2004, 2005). Schapira and Boutika (2012) provided an insight of the situation and the national authorities responded as follows: "Over the 10-year period from 1992 to 2002, there was a dramatic reduction in malaria transmitted by brackish water breeders in the Vietnamese part of the Mekong delta. The reductions could be ascribed to high levels of coverage with *ITNs* and widespread availability of treatment with artemisinin derivatives. Desalination may also have played a role. Between 1992-2001, at a cost of 12 billion US dollars, tidal floodgates were installed on the major rivers and canals and secondary canals were dredged in an effort to prevent seawater intrusion into the low-laying Ca Muu Peninsula. The purpose was to improve agricultural productivity (White, 2009); as a side effect, it may have helped reduce the malaria risk".

4.2 House design

Farming huts in the GMS usually have no walls or just one or two loose thatched bamboo or matting walls (Erhart *et al*, 2005; Ngo *et al*, 2008). The floors are loosely laid split bamboo slats or wooden boards; sleeping on a woven rattan mat offers some protection from mosquitoes entering from below, but many occupants sleep directly on the slat floor. A risk factor study in Phu Thuan village, Binh Phuoc Province, Viet Nam, showed that wooden or bamboo houses had a higher risk for malaria infection compared to cement houses (OR 4.18; 95% CI 1.45-12.10), and ethnic minority people living in traditional wooden/bamboo housing also had a higher risk for malaria (OR 5.57; p<0.01) compared with those living in cement housing (Abe *et al*, 2009). As slatted floors may provide entry for mosquitoes into houses in the same way as the eave gaps between the wall and the roof, there is a need to promote bed mats for bednet sleepers.

The literature on the impact of improved housing and screening against mosquitoes and vector-borne diseases in the GMS is scarce compared to African settings. A multivariate classification tree model analysis suggests that improved housing conditions might achieve a protective effect against malaria in poor rural areas of central Viet Nam (Thang *et al*, 2008) as it has been described elsewhere for other countries (Butraporn *et al*, 1986; Gamage-Mendis *et al*, 1991). Thang *et al* (2008) cited that the Vietnamese government's current poverty alleviation program (William and Huynh, 2005) consisting, in part, to provide brick houses for the poorest, might have a positive impact on malaria prevalence. People living in poor houses (incomplete or with walls and roofs made of palm thatch and mud) have a higher exposure to malaria than people occupying houses with complete brick and plaster walls, windows, and roofs.

House screening and permethrin-impregnated curtains were also found to reduce mosquito human biting rates as well as malaria infections in settings as diverse as the United States, Greece and Italy (Lindsay *et al*, 2002), Pakistan (Hewitt *et al*, 1996), rural parts of The Gambia (Kirby *et al*, 2009), East Africa (Kirby *et al*, 2008), urban Dar es Salam (Ogoma *et al*, 2009) and in rice irrigation scheme, Kenya (Atieli *et al*, 2009). With current efforts focused on malaria elimination and eventual eradication, there is an urgent need to "*engage policy makers in active advocacy of mosquito-proofing houses as one of the tools for integrated control........*" (Ogoma *et al*, 2009).

5. Personal protection and repellents

While travel advisories regarding the use of insect repellents such as DEET and permethrin-impregnated clothing and bednets are available (<u>http://www.mekongriverboats.com/</u> <u>guide_detail/9/56/Practical_Information/Insect_Precautions_on_board_Mekong_Cruises.</u> <u>html</u>), data on their effectiveness and usage are limited. The Thailand Bureau of Vector-Borne Diseases and national malaria control programs of Cambodia and Viet Nam provide LLIHs and repellents to special-at-risk populations (PMI, 2012).

The use of personal protection through mosquito coils and indigenous materials (oils, smoke, etc) is variable in GMS countries (Vythilingam *et al*, 2005a; Van Benthem *et al*, 2006; Moore *et al*, 2008; Tipmontree *et al*, 2009). Usage will vary according to availability, knowledge, occupation, education, income and location. Where an individual may use a coil in his/her village dwelling he/she may not do so when sleeping at home, in the forest or field for work purposes (Moore *et al*, 2008). These methods are widely practiced in agricultural communities but are of doubtful effectiveness in terms of protection against transmission,

although they often provide some relief from the nuisance aspect in situations of high mosquito (including vector) biting densities (WHO, 2006b). As poorer farming communities can ill afford to buy commercial repellents NGOs and partners are providing free repellents for personal protection and targeting migrants or local resident household with family members going temporarily into the forest (Networks, 2012).

A recent field trial conducted in Lao PDR showed that 15% DEET reduced the biting rate of mosquitoes over five hours by 99% and concentrations as low as 10% DEET have a 95% reduction in biting (DeRaedt, 2010). As this study was biased by a mixture of 50% DEET combined with untreated lotion, future work involving different concentrations of DEET should be sourced from the same supplier. Another confounder was the excessive quantity of DEET lotion which caused a layering effect on the arms and legs of the collectors (DeRaedt, 2010).

Experimental hut studies show that pyrethrum coils and permethrin-impregnated curtains reduced blood feeding of *An. stephensi* by 36% and 65%, respectively (Hewitt *et al*, 1996), but evidence on the impact of burning insecticide-containing mosquito coils in preventing malaria is lacking (Lawrence and Croft, 2004). Pyrethrum coils have a number of limitations: they are protective throughout the night; they require a heat source to vaporize the active ingredient, commonly a synthetic pyrethroid such as d-allethrin or parallethrin; they produce a visible and sometimes unpleasant smoke when ignited; and they tend to be brittle and can break in transit and use. Controlled studies in a closed room (92.8 cm³) showed that the continuous ignition of a certain weight of coil containing d-allethrin 0.3% w/w had a repellent effect and prevented bites of *Aedes aegypti* at a coverage radius of 7 m from the coil (Komalamisra *et al*, 2003). The addition of a synergist or a high concentration (0.5%) of dl, d-T80-allethrin, a conventional pyrethroid, that is twice the conventional dosage is effective against insectary-raised *An. dirus* (Chon Buri strain from Thailand) but not against *Ae. aegypti* (Katsuda *et al*, 2008).

A novel concept of spatial repellents to create a vector-free space, thereby preventing contact between human and vector, thus preventing disease transmission, is an attractive one especially for special at-risk populations in areas inaccessible to IRS or LLINs. The ideal spatial repellent is one that can be used under semi-walled shelters in rural village and farm plot settings. With current efforts focusing in malaria elimination (APMEN, 2012), there is considerable interest in vectors with behaviors that are not controlled by conventional IRS and ITNs. These "difficult" vectors will become the focus of residual transmission and will be the barrier to success or failure (Ferguson *et al*, 2010; Achee *et al*, 2012). Given that current tools are inadequate, Achee *et al* (2012) argued for the role of combined organized vector control (IRS and ITN) with personal protection (consumer products)

to enhance human protection from infection and where spatial repellents could equally be useful.

In presenting current evidence supporting the value of spatial repellents, Achee *et al* (2012) listed the benefits of sub-lethal over more conventional lethality-directed chemical approaches as follows: "1) marketable for insecticide-management purposes because it is useful for delaying the onset of resistance to active ingredients used for ITNs or IRS; 2) effective for outdoor protection, something that IRS and ITNs have little impact on; 3) useful in attacking other components of vector behavior such as pre-, during and posthost-seeking, *ie*, to disrupt critical behavioral sequences that can prevent blood-feeding (and disease transmission) and strengthen the effectiveness of integrated vector control strategies; 4) employable against multiple vectors, behaviors and species – not just those that feed and rest inside houses - and subsequently against other arthropod-borne diseases, and 5) useful against economically important insects, especially agricultural pests, where market forces will fuel the cost of active ingredient discovery and development."

CHALLENGES AND GAPS

The results from the numerous publications, reports and papers used in this review highlight the need for a detailed understanding of the distribution, species composition, behavior and insecticide resistance levels of Mekong vectors in order to successfully control malaria. Until the feeding behavior and vectorial status of each species/sibling species can be elucidated across their ranges, taking into account seasonal influences (an impossibly ambitious task with the tools currently available), then vector control activities will need to continue targeting entire species complexes based on their local overall behavioral characteristics. These considerations are important in the light of the goal of the GMS and WHO's Global Malaria Programme to eliminate malaria as a public health problem.

A serious challenge is that the investment in discovering and validating new tools and strategies has been minimal. Thirty years ago, it was recognized that the vector systems related to forest-like environments are relatively refractory to IRS, and that ITNs will provide the solution (Ismail *et al*, 1974, 1975; Chareonviriyaphap *et al*, 2000). These systems are characterized by *Anopheles* species complex and sibling species exhibiting differences in bionomics, phenology and vector competencies which have an important bearing on malaria transmission dynamics. Many control programs are recognizing that a single strategy for an entire country, and even for a single province/district is not applicable. This requires that situation-specific and at times, even species-specific strategies need to be employed (WHO SEARO, 2007).

Given that almost all the important malaria vectors in the GMS have been identified as species complexes, it is essential for public health entomologists and research scientists to collaboratively map the distribution of the sibling species of medically important complexes and to establish the role of each sibling species in malaria transmission and monitor their response to control measure. Gaining access to a regional molecular laboratory facility or real time PCR laboratory is a challenge and, until this is addressed, vector control activities will continue targeting entire species complexes based on text book information or local/ generalized behavioral characteristics. To emphasize the importance of identifying species complexes and for further research on these complexes, it is pertinent to reproduce the recommendations from an informal consultancy meeting on "Malaria vector species complexes and intra-specific variations: Relevance for malaria control and orientation for further research" held in Bangkok in November 1984 (WHO SEARO, 2007). These are as follows:

• "In order to simplify epidemiological mapping and stratification at the country level, as part of the program planning process, attempts should be made to link genetically-determined sibling species and associated variants with topographical and vegetational indicators".

• "Species distribution does not recognize political boundaries, and it is strongly recommended that collaboration between countries and regions be encouraged in investigations involving species complexes of malaria vectors".

Large scale entomological and mapping surveys similar to Garros *et al* (2008) in Viet Nam are urgently required in GMS countries to molecularly identify the different members of the important Minimus, Dirus, Maculatus, Aconitus, Hyrcanus and Barbirostris Subgroups and complexes, and to clarify the precise distributions of each sibling species. The practical applications of precise and accurate geographic distributions of vector species for malaria control programs are: *"1) an adequate choice of zones where vector control actions should focus; 2) a better selection of future study sites for entomologists working on secondary or local vector species; 3) studies on anopheline biodiversity relating to environmental and climatic changes; 4) analyses of landscape – species associations, and 5) modeling malaria risk maps or comparison of predictive ecological maps with field observations" (Garros <i>et al*, 2008).

More recently, the RBM Work stream on outdoor malaria transmission pointed out that LLINs will not control vectors biting before and after sleeping time and IRS will not control exophagic mosquitoes (RBM, 2012). Investments in new tools addressing outdoor transmission prioritizing the general population and for special risk groups are needed. Operational research will generate the evidence for the efficacy and feasibility of personal protective measures such as LLIHs, insecticide-treated clothing or spatial repellents. Many of the research topics were discussed among GMS countries and stakeholders in a malaria operational

research symposium in 2010 (USAID, 2010). The challenge is to advocate and mobilize resources (*eg*, identify existing networks and potential resources and develop costed plan) for outdoor transmission and the network (RBM, 2012).

Recent work in the GMS has established a baseline for the major malaria vectors across much of the Mekong region, showing large differences among species and countries, but there is a need for follow-up of resistance monitoring. In 2011, during the Malaria Programme Managers' meeting held in Manila, WPRO and SEARO proposed to establish an Asia Pacific Vector Resistance Network (APVRN) to support insecticide resistance monitoring through WHO (WPRO and SEARO) and to strengthen the resistance monitoring and standarding methodologies and reporting system. Recent international and biregional workshops on outdoor transmission (RBM, 2011, 2012) and monitoring insecticide resistance and mapping of malaria vectors in the GMS (WHO WPRO, 2012) produced several key recommendations and actions. The priority research questions extracted from a RBM (2011) meeting were as follows:

Vector bionomics

a. What is the bionomics and distribution of *An. dirus* at local scale taking into account seasonal and environmental changes (including mapping, land use/cover, etc)?

b. What is the role of secondary vectors in maintenance of transmission on Pf and Pv in different environmental settings (including mapping, land use/cover, etc)?

• Prevention measures

a. What innovative or existing personal protection methods or tools beyond nets are effective in preventing bites of malaria vectors in different target groups?

b. What is the best policy for replacement of LLINs and re-impregnation of ITNs, including waste management, net integrity and bioefficacy?

c. What is the acceptability of different types of LLINs/ITNs and personal protective measures?

The general consensus (and a research priority) is the evaluation of well designed trials of innovative strategies in intractable and difficult situations. One challenge is how to better coordinate, share and disseminate findings gathered from field trials, updates and small-scale, modest studies. The latter may include but not be restricted to studies on vector (primary and secondary) distribution related to environmental changes, vector susceptibility status to insecticides and vector behavior relevant to transmission. Entomologists in the GMS are challenged to pay more attention to understanding the various causal relations better, moving from mosquito inventories and checklists to detailed analysis of interactions for example between physiology, environment, and bionomics.

Another challenge is to strengthen national capacity to plan and implement routine monitoring of insecticide resistance and management as outlined in the GPIRM including allocation of adequate resources to implement the proposed national plans. As baseline data from previous sentinel sites in the MALVECASIA project are available, consolidation of existing data and collection of data from new sites should be considered. The extent of variation in resistance frequency within small areas needs to be assessed. Due to the focal nature of resistance and the limited representation or coverage of sentinel surveillance, priority sites should therefore be those in which the threat of resistance appears to be greatest, for example, those with the greatest malaria incidence and where insecticides are used in large quantities for both public health and agriculture. In order to establish the geographical spread of resistance, the GPIRM recommended a further detailed study in the area where a newly identified focus of resistance is identified (WHO, 2012b). As continuing use of LLINs is likely to contribute significantly to selection pressure for resistance, WHO (2012) has advised frequent entomological monitoring, at least once a year and preferably every 6 months. In areas of high coverage with LLINs, pyrethroids should not be used for IRS, as this will contribute to selection pressure. IRS should therefore be done with alternative, non-pyrethroid insecticides. The alternative insecticides should preferably be used in rotations scheme to avoid the development of resistance in any one of them. There is a need to strengthen countries' capacity to manage, analyze and interpret data for national decision-making-in particular for revising vector control plans to take into account insecticide resistance management.

CONCLUSIONS

This review attempts to consolidate the entomological studies from 2003 to 2012 in a regional context. Sharing entomological data in this way is a rational starting point for building a regional *Anopheles* vector database that will facilitate concerted planning and action to improve malaria control and elimination. Of paramount importance is the development of a regional database on vector resistance to insecticides and GMS countries are in the process to establish the Asia Pacific Malaria Vector Resistance Network for monitoring and reporting for effective management of insecticide resistance which is coordinated by the Asian Collaborative Training Program for Malaria (ACTMalaria).

More efforts are needed to go beyond simply bednet protection in order to achieve malaria elimination in GMS countries (RBM, 2012). Innovative research and programming on reducing outdoor transmission of malaria in various eco-epidemiological settings such as villages, farm huts, forest fringes, forests, orchards, commercial plantations and coastal areas will provide better evidence for action and planning. This may include LLIN and IRS

combinations, durable insecticidal wall linings, and support for new areas of intervention and research. The era of mass distribution is over and new methods of achieving continuous distribution are needed. Well-designed LLIN durability studies are also needed as the lifespan of particular brands makes a big difference in the cost-effectiveness of any procurement, which is not being considered currently in any purchasing decisions by funding agencies. At present, the policy of considering the cheapest purchase price in their tendering process is not based on scientific evidence of durability, bio-efficacy and consumer preference data (RBM, 2012).

Although GMS is leading the way with a significant work program on outdoor malaria transmission, there is a lack of strategies for the control of outdoor biting vectors, and there are enormous knowledge gaps regarding their efficacy, effectiveness, feasibility and acceptability.

By their very nature, Mekong vectors are efficient, adaptable and elusive compared to other *Anopheles* species, and will in time, be replaced by probably less or more efficient species or non-vectors in changing landscapes, potentially causing significant public health problems where this occurs. There is a need to monitor these trends and coordinate, share and disseminate findings from small-scale, modest studies to well-designed large scale studies. With better coordination of studies and protocols, there is an opportunity to update the knowledge base, compare data and extend the scope of individual projects (RBM, 2012). Corrective actions are urgently needed to better represent the entomological perspective on malaria control and to improve and enrich the evidence base for malaria control strategies.

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