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SUSCEPTIBILITY OF TWO KARYOTYPIC FORMS OF *Anopheles aconitus* (DIPTERA: CULICIDAE) TO *Plasmodium falciparum* AND *P. vivax*

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SUMMARY

Four laboratory-raised colonies of two karyotypic forms of *Anopheles aconitus*, i.e., Form B (Chiang Mai and Phet Buri strains) and C (Chiang Mai and Mae Hong Son strains), were experimentally infected with *Plasmodium falciparum* and *P. vivax* using an artificial membrane feeding technique and dissected eight and 12 days after feeding for oocyst and sporozoite rates, respectively. The results revealed that *An. aconitus* Form B and C were susceptible to *P. falciparum* and *P. vivax*, i.e., Form B (Chiang Mai and Phet Buri strains/*P. falciparum* and *P. vivax*) and Form C (Chiang Mai and Mae Hong Son strains/*P. vivax*). Comparative statistical analyses of the oocyst rates, average number of oocysts per infected midgut and sporozoite rates among all strains of *An. aconitus* Form B and C to the ingroup control vectors, *An. minimus* A and C, exhibited mostly no significant differences, confirming the high potential vector of the two *Plasmodium* species. The sporozoite-like crystals found in the median lobe of the salivary glands, which could be a misleading factor in the identification of true sporozoites in salivary glands were found in both *An. aconitus* Form B and C.

KEYWORDS: *Anopheles aconitus*; Karyotypic form; Susceptibility; *Plasmodium falciparum*; *P. vivax.*

INTRODUCTION

Malaria remains a major health problem of the world, particularly, in the tropical[41]. In Thailand, four species of malaria parasites are found; the most common species are *Plasmodium vivax* (52.50%) and *P. falciparum* (45.89%), while *P. malariae* (0.32%) and *P. ovale* (one case reported from Chiang Mai province in 1996) are rare, and 1.29% are mixed infections[2]. The disease is generally limited to rural communities living in and near forested areas, mountains and foothills, particularly, those residing in newly opened land settlements of semi-forested areas earning their living by growing agricultural crops, and in the areas near and along the borders with the neighboring countries of Kampuchea, Laos, Myanmar and Malaysia[1,2].

So far, at least six anopheline species have been incriminated as primary and secondary vectors of malaria in Thailand. The primary vectors are *Anopheles dirus* Peyton & Harrison, *An. minimus* Theobald, and *An. maculatus* Theobald[3,29,31,39]. The taxa of above three vectors are all species complexes, and the members of each complex cannot be easily distinguished from each other[4,6,15]. The secondary vectors are *An. sundaicus* (Rodenwaldt), *An. aconitus* Donitz, and *An. pseudowillmori* (Theobald), one of the member species of *An. maculatus* complex[8,16,17,31]. For *An. aconitus*, it was also incriminated as a vector of malaria in other countries, i.e., Indonesia[20,21], Bangladesh[22], Malaysia[24] and India[23].

As early as 1944, *An. aconitus* was considered a primary vector of malaria in Thailand[33]. However, such implications lacked confirmation until GOULD *et al*.[17] found one *An. aconitus* female positive for both oocysts and sporozoites, and another one positive for only oocysts by dissection in the rice plain just north of Bangkok in April and August, respectively. In addition, the human-baited, whom bitten during April was subsequently got infection with *P. vivax*. This area was known to be endemic for *P. vivax* essentially to the exclusion of all other *Plasmodium* species. Thus, the authors concluded that *An. aconitus* was obviously the vector. Additional positive specimens of *An. aconitus* have not been reported in Thailand up to this time, except the reports of positive ELISA for circumsporozoite (CS) antigens from southern Thailand[25]. Recently, three karyotypic forms of *An. aconitus*, i.e., Form A (X_1, X_2, Y_1), B (X_1, X_2, Y_2), and C (X_1, X_2, Y_3) have been incriminated sympatrically from northern Thailand, while Form D (X_3, X_4, Y_4) has been reported from only Java, Indonesia[5]. Apparently, little is known about the vector potential of *An. aconitus* in northern Thailand, particularly among the karyotypic forms, which is intensively needed to confirm its vector status. Hence the present study reports the susceptibility of *An. aconitus* Form B and C strains from Chiang Mai

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province (northern Thailand), Mae Hong Son province (northwest Thailand), and Phet Buri province (southwest Thailand) to *P. falciparum* and *P. vivax.*

MATERIALS AND METHODS

Laboratory-raised *An. aconitus* Form B and C: Three provinces, the endemic areas of malaria in Thailand, i.e., Chiang Mai (Ban Pang Mai Daeng, Maetang district), Mae Hong Son (Ban Huai Pong Kan, Muang district) and Phet Buri (Ban Tha Salao, Nong Ya Plong district), the same localities as the previous studies by JUNKUM *et al*.[19], were the sites for mosquito collections by using both human-baited and buffalo-baited traps. Four laboratory-colony strains of *An. aconitus* were established based on metaphase karyotypes and localities. Since the results of metaphase karyotype identifications of 3, 4 and 89 iso-female lines (isolines) of *An. aconitus* strains from Mae Hong Son, Phet Buri and Chiang Mai, respectively, using the techniques of CHOOCHOTE *et al*.[11], revealed the two karyotypic forms, i.e., Form B (X_1, X_2, Y_2), and C (X_1, X_2, Y_3) (detailed descriptions of karyotypic forms were in the former studies by JUNKUM *et al*.[19]). Thus, two colonies of Form B, Phet Buri and Chiang Mai strains, were established by pooling 4 and 20 isolines, respectively. Two colonies of Form C, Mae Hong Son and Chiang Mai strains, were established by pooling 3 and 20 isolines, respectively. These colonies were successfully reared by using the method of CHOOCHOTE *et al*.[12] in an insectary room at 27 ± 2 ºC, 70-80% RH, illuminated with a combination of natural daylight from glass-window and fluorescent lighting (approximately 12 h a day) for more than five consecutive generations, and were used for malarial susceptibility test throughout the experiments.

Outgroup and ingroup control vectors: The outgroup, *An. dirus* B, a species member of *An. dirus* complex belongs to the *leucosphyrus* group, and the ingroup, *An. minimus* A and C, the same taxon as *An. aconitus* Form B and C in the Myzomyia series, were used as the control vectors in the malarial susceptibility experiments. *An. dirus* B was obtained originally from Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, and the free-mating colony was estabilised in the insectarium of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand for more than two decades. The free-mating colony of *An. minimus* A (CM strain) was from northern Thailand[36]. The CM strain was confirmed as species A by metaphase karyotypes and DNA sequence analysis of the D3 region of rDNA[37]. For artificial mating colony of *An. minimus* C, it was established by pooling 10 isolines of *An. minimus* C strain from Kanchanaburi province, central Thailand. All 10 isolines were identified to species C by using the combination characteristics of adults[38] and metaphase karyotypes[5]. Subsequently, additional evidence of species C was confirmed by DNA sequences of D3 region[32].

***P. falciparum* and *P. vivax* gametocytes:** The gametocytes of *P. falciparum* and *P. vivax* were obtained from malaria patients, whom got infection from Maetang and/or other districts in Chiang Mai province. Ten mL of blood containing gametocytes of the above malaria species were collected by venepuncture into a heparinized syringe, kept in ambient temperature[34], and used for infection of mosquitoes within 12 h.

Infection of mosquitoes with *P. falciparum* and *P. vivax* gametocytes: After emergence, all adult female mosquitoes were provided with 5% sucrose solution until age of 4-6 days, subsequently, they were fasted for 12 hours prior to the infections. The 12-hours fasted females of *An. aconitus* Form B and C, outgroup control mosquito-vector (*An. dirus* B), and ingroup control mosquito-vectors (*An. minimus* A and C) were put in a paper cup size 8.5 cm in diameter and 11 cm in depth (50 fasted females per cup for each species), and allowed to feed on heparinized blood containing gametocytes (gametocyte density of *P. falciparum* = 21 per 1 µL; *P. vivax* = 28, 17 and 34 per 1 µL in experiment 1, 2 and 3, respectively) using artifial membrane feeding techniques as described by CHOMCHARN *et al*.[10] The fully engorged females were separated to small paper cups (diameter 6.5 cm, depth 8 cm) with 10 mosquitoes per cup and maintained in an incubator at 27 ± 2 °C, 70-80% RH. Cotton wool pad soaked with 5% sucrose solution was provided regularly and changed every other day until the time of dissections. Eight and twelve days after feeding, the infected mosquitoes were dissected and examined for oocysts in midguts and sporozoites in salivary glands, respectively.

RESULTS

Oocyst rates of *An. dirus* B, *An. minimus* A and C, and *An. aconitus* Form B and C: Details of oocyst rates are shown in Table 1. Observations on dissected midguts eight days after feeding revealed that *An. aconitus* Form B were susceptible to both *P. falciparum* and *P. vivax*, and Form C was susceptible to *P. vivax* . The 100% oocyst rates and 5.22 – 126.18 average number of oocysts per infected midgut obtained from *An. dirus* B, the outgroup control mosquito-vector, indicated the all feedings were conditional experiments, which reflected on the proper density and maturity of infective gametocytes in infected blood.

In the experimental feedings of *P. falciparum*, the oocyst rates and average number of oocysts per infected midgut of *An. aconitus* Form B (Chiang Mai and Phet Buri strains) did not differed significantly ($p > 0.05$) from the ingroup control-vector, *An. minimus* A. Similar results also were obtained from statistical analysis of the oocyst rates and average number of oocysts per infected midgut between *An. aconitus* Form B strains from Chiang Mai and Phet Buri provinces.

In the experimental feedings of *P. vivax*, mostly, the oocyst rates and average number of oocysts per infected midgut of *An. aconitus* Form B (Chiang Mai and Phet Buri strains) and C (Chiang Mai and Mae Hong Son strains) did not differ significantly ($p > 0.05$) from *An. minimus* A and C, except the average number of oocysts per infected midgut of *An. aconitus* Form C (Chiang Mai strain: experiment 1) was significantly less than that in *An. minimus* A, and Form C (Mae Hong Son strain: experiment 2) was significantly greater than that in the *An. minimus* A. Similar results also were recovered from statistical analysis of the oocyst rates and average number of oocysts per infected midgut between *An. aconitus* Form B (Chiang Mai strain) and C (Chiang Mai and Mae Hong Son strains) in experiment 1 and 2, except for only the average number of oocysts per infected midgut of *An. aconitus* Form C (Mae Hong Son strain) was significantly greater than that in the Form B (Chiang Mai strain) in experiment 2.

Oocyst and sporozoite rates of *An. dirus* B, *An. minimus* A and C, and *An. aconitus* Form B and C: Details of oocyst and sporozoite rates are shown in Table 2. The dissection of midguts of *An. dirus* B, *An. minimus* A and C, and all strains of *An. aconitus* Form B and C 12

Table 1
The oocyst rates of *An. dirus* B, *An. minimus* A and C and *An. aconitus* Form B and C after feeding on blood containing gametocytes of *P. falciparum* and *P. vivax*, all dissected 8 days after feeding

Malaria species	Mosquito species						
	An. dirus B	*An. minimus*		*An. aconitus* Form			
		A	C	B		C	
				CM	PB	CM	MS
P. falciparum							
Oocyst rate (No.)	100 (20/20)	91.67 (11/12)	ND	62.50 (5/8) NS	80.00 (8/10) NS	ND	ND
Average No. oocysts per Infected midgut (range)	84.75 ± 45.53 (19-182)	18.64 ± 22.61 (1-81)	ND	31.40 ± 21.85 NS(2-35)	19.63 ± 25.14 NS(1-79)		
P. vivax							
Experiment 1							
Oocyst rate (No.)	100 (7/7)	100 (5/5)	ND	100 (5/5)	ND	60.00 (3/5) NS	ND
Average No. oocysts per Infected midgut (range)	69.71 ± 26.82 (39-118)	18.00 ± 6.93 (11-27)	ND	10.00 ± 6.04 (4-19)	ND	4.33 ± 4.93 * (1-10)	ND
Experiment 2							
Oocyst rate (No.)	90.00 (9/10)	83.33 (5/6)	ND	66.67 (4/6) NS	ND	ND	66.67 (4/6) NS
Average No. oocysts per Infected midgut (range)	5.22 ± 3.73 (1-11)	2.00 ± 1.41 (1-4)	ND	1.00 ± 0.00 (1)	ND	ND	6.00 ± 3.46 * (3-9)
Experiment 3							
Oocyst rate (No.)	100 (11/11)	81.82 (9/11)	50.00 (5/10) NS	ND	50.00 (2/4) NS	ND	ND
Average No. oocysts per Infected midgut (range)	126.18 ± 55.92 (34-223)	22.78 ± 15.18 (2-54)	7.80 ± 8.23 NS(1-22)	ND	19.50 ± 12.02 NS(11-28)	ND	ND

Mosquito strain; CM: Chiang Mai, MS: Mae Hong Son, PB: Phet Buri; Oocyst rate: NS, $p > 0.05$, *, $p < 0.05$ (Fisher exact test); Average No. oocysts per infected midgut: NS, $p > 0.05$, * , $p < 0.05$ (t-test, two-sided); ND: not done.

days after feeding on blood containing *P. falciparum* and *P. vivax* gametocytes revealed that the oocyst rates were 95% (*An. aconitus* Form B: Phet Buri strain), 96.30% (*An. aconitus* Form B: Chiang Mai strain), 100% (*An. minimus* A), and 100% (*An. dirus* B), for *P. falciparum*, and 14.28-75% (all strains of *An. aconitus* Form B and C), 39.13% (*An. minimus* C), 11.11-100% (*An. minimus* A) and 15.79-100% (*An. dirus* B) for *P. vivax.* Statistical analyses of the oocyst rates among the ingroup control mosquito-vectors, *An. minimus* A and C, and all strains of *An. aconitus* Form B and C were not done because at this period (12 days of postblood meal) the mature oocysts from the midgut of the control vectors ruptured and yielded unreliable results. Nonetheless, the satisfactory percentages of oocyst rates obtained from both outgroup and ingroup control-vectors were confirmed the conditional experiments.

The dissection of salivary glands 12 days after feeding demonstrated that *An. aconitus* Form B strains from Chiang Mai and Phet Buri were efficiently potential vectors for *P. falciparum*, and Form B strains from Chiang Mai and Phet Buri and Form C strains from Chiang Mai and Mae Hong Son were the efficiently potential vectors for *P. vivax* when compared to the ingroup control-vectors, *An. minimus* A and C. Comparative statistical analyses of sporozoite rates among *An. minimus* A and C, and four strains of *An. aconitus* Form B and C of all experiments exhibited no significant differences ($p > 0.05$), except only *An. aconitus* Form B (Phet Buri strain) differed significantly ($p < 0.05$) in the experimental feeding of *P. falciparum.*

Another interesting point in the present study is the sporozoite-like crystal found in the median lobe of salivary glands of both *An. aconitus* Form B and C, i.e., Form B: Chiang Mai strain 3.70% (1/27), Phet Buri strain 20% (4/20) (experimental feeding on *P. falciparum*); Form C: Mae Hong Son strain 28.57% (4/14) (experimental feeding on *P. vivax*). The sporozoite-like crystal rather resembles a true sporozoite, particularly, when it is inside a non-squashed salivary glands. The latter has regular spindle-shaped while the former has irregular, long or short with blunt or tapered end(s) (Fig. 1). It was stable in 0.85% normal saline solution for at least half an hour and after that the dissolve of the crystal could be obviously seen, and could be easily distinguished from the true sporozoite.

DISCUSSION

In order to incriminate a mosquito vector in an endemic area of mosquito-borne human diseases, it is necessary to confirm the

Table 2
The oocyst and sporozoite rates of *An. dirus* B, *An. minimus* A and C and *An. aconitus* Form B and C after feeding on blood containing gametocytes of *P. falciparum* and *P. vivax*, all dissected 12 days after feeding

Malaria species	Mosquito species						
	An. dirus B	*An. minimus*		*An. aconitus* Form			
		A	C	B		C	
				CM	PB	CM	MS
P. falciparum							
Oocyst rate (No.)	100 (23/23)	100 (11/11)	ND	96.30 (26/27)	95.00 (19/20)	ND	ND
Average No. oocysts per	85.00 ± 26.41	5.45 ± 3.86	ND	13.35 ± 15.60	28.58 ± 28.42	ND	ND
Infected midgut (range)	(39-145)	(1-15)		(1-54)	(2-90)		
Sporozoite rate (No.)	95.65 (22/23)	100 (11/11)	ND	70.37 (19/27) NS	45.00 (9/20) *	ND	ND
P. vivax							
Experiment 1							
Oocyst rate (No.)	100 (4/4)	100 (2/2)	ND	ND	ND	100 (4/4)	ND
Average No. oocysts per	28.00 ± 25.07	5.50 ± 4.95	ND	ND	ND	9.75 ± 4.03	ND
Infected midgut (range)	(6-64)	(2-9)				(5-14)	
Sporozoite rate (No.)	100 (4/4)	100 (2/2)	ND	ND	ND	100 (4/4)	ND
Experiment 2							
Oocyst rate (No.)	15.79 (3/19)	11.11 (1/9)	ND	16.67 (2/12)	ND	ND	14.28 (2/14)
Average No. oocysts per	1.33 ± 0.58	1.00 ± 0.00	ND	2.00 ± 1.41	ND	ND	1.00 ± 0.00
Infected midgut (range)	(1-2)	(1)		(1-3)			(1)
Sporozoite rate (No.)	80.00 (16/20)	33.33 (3/9)	ND	16.67 (2/12) NS	ND	ND	14.28 (2/14) NS
Experiment 3							
Oocyst rate (No.)	100 (17/17)	66.67 (12/18)	39.13 (9/23)	ND	75.00 (9/12)	ND	ND
Average No. oocysts per	33.53 ± 20.12	2.92 ± 1.24	2.89 ± 1.83	ND	2.89 ± 2.15	ND	ND
Infected midgut (range)	(7-77)	(1-5)	(1-6)		(1-7)		
Sporozoite rate (No.)	100 (17/17)	77.78 (14/18)	52.17 (12/23) NS	ND	66.67 (8/12) NS	ND	ND

Mosquito strain; CM: Chiang Mai, MS: Mae Hong Son, PB: Phet Buri; Sporozoite rate: NS, $p > 0.05$, *, $p < 0.05$ (Fisher exact test, χ^2-test for only experiment 3); ND: not done.

susceptibility rate in a laboratory-bred, clean mosquito colony that has been fed on a carrier blood containing pathogens[30]. Thus, by using this criterion, the susceptibility test in an experimental laboratory is still a useful tool when suspecting the potential vector of a certain mosquito species. Nevertheless, the susceptibility alone does not imply an important role in the transmission of disease in nature, whereas a refractory one can entirely rule out its significance. According to the vectorial status of *An. aconitus* to *P. falciparum* and *P. vivax* as

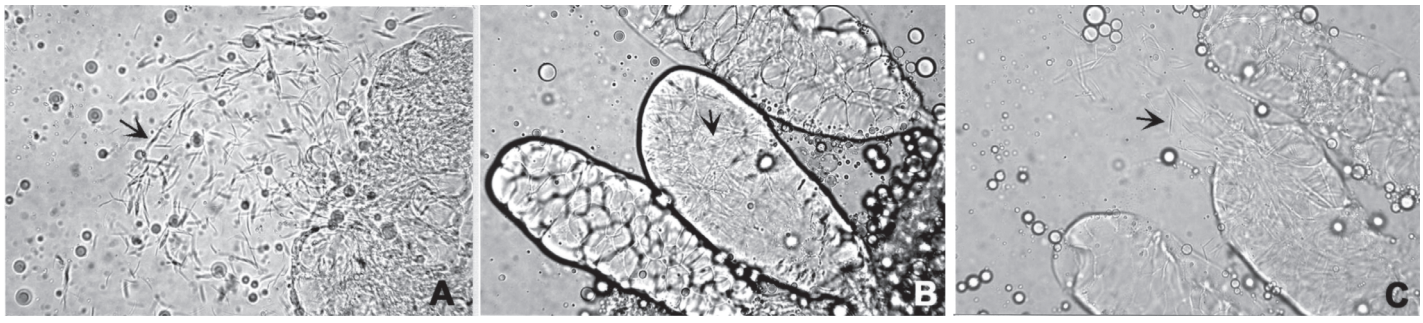

Fig. 1 - Salivary glands of *An. aconitus* Form B. (A) Showing free flow *P. vivax* sporozoites from the squashed salivary glands. Note, the regular spindle-shaped sporozoites (small arrow). (B) Showing sporozoite-like crystals inside the median lobe of salivary glands (small arrow). (C) Showing free flow sporozoite-like crystals from the squashed salivary glands. Note, the irregular, long or short, crystals with blunt or tapered end(s) (small arrow).

determined by the susceptibility tests using a laboratory-bred, clean mosquito colony have never been done and/or reported before this time. The high oocyst and sporozoite rates of *An. aconitus* Form B strains from Chiang Mai and Phet Buri provinces to infection with *P. falciparum*, and Form B strains from Chiang Mai and Phet Buri provinces and Form C strains from Chiang Mai and Mae Hong Son provinces to infection with *P. vivax* in the present study, confirming the secondary vector status of *An. aconitus* as reported by GOULD *et al.*[17]. Nonetheless, further investigations on the oocyst and sporozoite rates of wild-caught female *An. aconitus* in an endemic area of malaria in Chiang Mai province and/or other suspected areas should be done intensively to determine its role as a naturally transmissive vector.

Many Thailand *Anopheles* species have been reported positive ELISA for circumsporozoite (CS) antigens of *P. falciparum* and *P. vivax* by using the whole body and/or head and thorax of mosquitoes[7,13,14,18,25]. This diagnostic tool did not definitely incriminate the mosquito as the natural vector, since it could be detected CS protein from the developing oocysts[9], soluble CS protein shed from oocysts and sporozoites[40] and CS protein in various body parts[26]. In addition, false positive *P. falciparum* and *P. vivax* detections by ELISA were reported[35]. However, the mosquito species which were highly susceptible to malarial infections could not be incriminated as the potential vectors, since sporozoite did not invade salivary glands[28]. Judged from the above evidences, therefore, the combining of positive ELISA for CS antigens with sporozoite rate of a laboratory-bred, clean *Anopheles* colony should be the important evidences prior to the incrimination of potentially natural vector.

Additionally, the sporozoite-like crystal found in the median lobe of salivary glands of *An. aconitus* Form B and C might be one of the important, missed leading factor in the identification of true sporozoite in salivary glands of the laboratory susceptibility experiments and/or wild-caught *Anopheles* females. Similar results have been reported in *An. sinensis* Form A and B[27].

RESUMO

Suscetibilidade de duas formas cariotípicas de *Anopheles aconitus* (Diptera: Culicidae) a *Plasmodium falciparum* e *P. vivax*

Quatro colônias desenvolvidas em laboratório, de duas formas cariotípicas de *Anopheles aconitus* i.e. forma B (cepa Chiang Mai e Phet Buri) e C (Cepa Chiang Mai e Mae Hong Son), foram infectadas experimentalmente com *Plasmodium falciparum* e *P. vivax* usando técnica de alimentação com membrana artificial e dissecados oito e 12 dias após alimentação da média de oocistos e esporozoitos, respectivamente. Os resultados revelaram que *An. aconitus* formas B e C foram suscetíveis ao *P. falciparum* e *P. vivax* isto é, forma B (cepa Chiang Mai e Phet Buri/*P. falciparum* e *P. vivax)* e forma C (cepa Chiang Mai e Mae Hong Son/*P. vivax).* Análises estatísticas comparativas das taxas de oocistos, número médio de oocistos por intestino médio infectado e taxas de esporozoitos entre todas as cepas de *An. aconitus* formas B e C ao grupo interno de vetores controles, *An. minimus* A e C, não exibiram nenhuma diferença significante, confirmando o alto potencial vetor das duas espécies de *Plamodium.* Os cristais semelhantes a esporozoitos encontrados no lobo médio das glândulas salivares que poderiam ser um fator enganoso na identificação de esporozoitos verdadeiros nas glândulas salivares foram encontrados em ambos *An. aconitus* formas B e C.

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REFERENCES

1. ANNUAL REPORT - Thailand, Division of Malaria, Department of Communicable Disease Control, Ministry of Public Health, 1998.

2. ANNUAL REPORT - Thailand, Division of Malaria, Department of Communicable Disease Control, Ministry of Public Health, 2002.

3. AYURAKITKOSOL, L. & GRIFFITH, M.E. - Progress toward malaria eradication in Thailand. In: PROCEEDINGS OF THE PACIF. SCI. CONGRESS, 9., Bangkok, 1957. v. 17, p. 122-136, 1963.

4. BAIMAI, V.; GREEN, C.A.; ANDRE, R.G.; HARRISON, B.A. & PEYTON, L.E. - Cytogenetic studies of some species complexes of *Anopheles* in Thailand and Southeast Asia. **Southeast Asian J. trop. Med. publ. Hlth., 15:** 536-546, 1984.

5. BAIMAI, V.; KIJCHALAO, U. & RATTANARITHIKUL, R. - Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. V. The Myzomyia series, Subgenus *Cellia* (Diptera: Culicidae). **J. Amer. Mosq. Contr. Ass., 12:** 97-105, 1996.

6. BAIMAI, V.; KIJCHALAO, U.; RATTANARITHIKUL, R. & GREEN, C.A. - Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. II. Maculatus group, Neocellia series, Subgenus *Cellia*. **Mosq. Syst., 25:** 116-123, 1993.

7. BAKER, E.Z.; BEIER, J.C.; MEEK, S.R. & WIRTZ, R.A. - Detection and quantification of *Plasmodium falciparum* and *P. vivax* infections in Thai-Kampuchean *Anopheles* (Diptera: Culicidae) by enzyme-linked immunosorbent assay. **J. med. Entomol., 24:** 536-541, 1987.

8. BARNES, M.E. - Notes on the anopheline mosquitoes of Siam. **Amer. J. Hyg., 3:** 121-126, 1923.

9. BEIER, J.C.; PERKINS, P.V.; WIRTZ, R.A. *et al* - Field evaluation of an enzyme-linked immunosorbent assay (ELISA) for *Plasmodium falciparum* sporozoite detection in anopheline mosquitoes from Kenya. **Amer. J. trop. Med. Hyg., 36:** 459-468, 1987.

10. CHOMCHARN, Y.; SURATHIN, K.; BUNNAG, D.; SUCHARIT, S. & HARINASUTA, T. - Effect of a single dose of primaquine on a Thai strain of *Plasmodium falciparum.* **Southeast Asian J. trop. Med. publ. Hlth, 11:** 408-412, 1980.

11. CHOOCHOTE, W.; PITASAWAT, B.; JITPAKDI, A. *et al.* - The application of ethanol-extracted *Gloriosa superba* for metaphase chromosome preparation in mosquitos. **Southeast Asian J. trop. Med. publ. Hlth, 32:** 76-82, 2001.

12. CHOOCHOTE, W.; SUCHARIT, S. & ABEYEWICKREME, W. - A note on adaptation of *Anopheles annularis* Van Der Wulp, Kanchanaburi, Thailand, to free mating in a 30x30x30 cm cage. **Southeast Asian J. trop. Med. publ. Hlth, 14:** 559-560, 1983.

13. FRANCES, S.P.; KLEIN, T.A.; WIRTZ, R.A. *et al*. - *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins in anophelines (Diptera: Culicidae) collected in eastern Thailand. **J. med. Entomol., 33:** 990-991, 1996.

14. GINGRICH, J.B.; WEATHERHEAD, R.A.; SATTABONGKOT, A.; PILAKASIRI, J. & WIRTZ, R.A. - Hyperendemic malaria in a Thai village: dependence of year-round transmission on focal and seasonally circumscribed mosquito (Diptera: Culicidae) habitats. **J. med. Entomol., 27:** 1016-1026, 1990.

15. GREEN, C.A.; GASS, R.F.; MUNSTERMANN, L.E. & BAIMAI, V. - Population-genetic evidence for two species in *Anopheles minimus* in Thailand. **Med. vet. Entomol., 4:** 25-34, 1990.

16. GREEN, C.A.; RATTANARITHIKUL, R.; PONGPARIT, S.; SAWADWONGPORN, P. & BAIMAI, V. - A newly-recognized vector of human malarial parasites in the Oriental region, *Anopheles* (*Cellia*) *pseudowillmori* (Theobald, 1910). **Trans. roy. Soc. trop. Med. Hyg., 85:** 35-36, 1991.

17. GOULD, D.J.; ESAH, S. & PRANITH, U. - Relation of *Anopheles aconitus* to malaria transmission in the central plain of Thailand. **Trans. roy. Soc. trop. Med. Hyg., 61:** 441-442, 1967.

18. HARBACH, R.E.; GINGRICH, J.B. & PANG, L.W. - Some entomological observations and malaria transmission in a remote village in Northwestern Thailand. **J. Amer. Mosq. Contr. Ass., 3:** 296-301, 1987.

19. JUNKUM, A.; JITPAKDI, A.; KOMALAMISRA, N. *et al*. - Comparative morphometry and morphology of *Anopheles aconitus* Form B and C eggs under scanning electron microscope. **Rev. Inst. Med. trop. S. Paulo, 46:** 257-262, 2004.

20. KIRNOWARDOYO, S. - Status of *Anopheles* malaria vectors in Indonesia. **Southeast Asian J. trop. Med. publ Hlth., 16:** 129-132, 1985.

21. KIRNOWORDOYO, S. & SUPALIN - Zooprophylaxis as useful tool for control of *Anopheles aconitus* transmitted malaria in central Java, Indonesia. **J. commun. Dis., 18:** 90-94, 1986.

22. MAHESWARY, N.P.; HABIB, M.A. & ELIAS, M. - Incrimination of *Anopheles aconitus* Donitz as a vector of epidemic malaria in Bangladesh. **Southeast Asian J. trop. Med. publ. Hlth, 23:** 798-801, 1992.

23. PRAKASH, A.; BHATTACHARYYA, D.R.; MOHAPATRA, P.K. & MAHANTA, J. - Role of the prevalent *Anopheles* species in the transmission of *Plasmodium falciparum* and *P. vivax* in Assam state, north-eastern India. **Ann. trop. Med. Parasit., 98:** 559-568, 2004.

24. RAHMAN, W.A.; ABU-HASSAN, A. & ADANAN, C.R. - Seasonality of *Anopheles aconitus* mosquitoes, a secondary vector of malaria, in an endemic village near the Malaysia-Thailand border. **Acta trop., 55:** 263-265, 1993.

25. RATTANARITHIKUL, R.; KONISHI, E. & LINTHICUM, K.J. - Detection of *Plasmodium vivax* and *Plasmodium falciparum* circumsporozoite antigen in anopheline mosquitoes collected in southern Thailand. **Amer. J. trop. Med. Hyg., 54:** 114-121, 1996.

26. ROBERT, V.; VERHAVE, J.P.; PONNUDURAI, T. *et al*. - Study of the distribution of circumsporozoite antigen in *Anopheles gambiae* infected with *Plasmodium falciparum*, using the enzyme-linked immunosorbent assay. **Trans. roy. Soc. trop. Med. Hyg., 82:** 389-391, 1988.

27. RONGSRIYAM, Y.; JITPAKDI, A.; CHOOCHOTE, W. *et al*. - Comparative susceptibility of two forms of *Anopheles sinensis* Wiedemann 1828 (Diptera: Culicidae) to infection with *Plasmodium falciparum*, *P. vivax*, *P. yoelii* and the determination of misleading factor for sporozoite identification. **Southeast Asian J. trop. Med. publ. Hlth, 29:** 159-167, 1998.

28. ROSENBERG, R. - Inability of *Plasmodium knowlesi* sporozoites to invade *Anopheles freeborni* salivary glands. **Amer. J. trop. Med. Hyg., 34:** 687-691, 1985.

29. ROSENBERG, R.; ANDRE, R.G. & SOMCHIT, L. - Highly efficient dry season transmission of malaria in Thailand. **Trans. roy. Soc. trop. Med. Hyg., 84:** 22-28, 1990.

30. SASA, M. - **Human filariasis: a global survey of epidemiology and control.** Tokyo, University of Tokyo Press, 1976.

31. SCANLON, J.E.; PEYTON, E.L & GOULD, D.J. - An annotated checklist of the *Anopheles* of Thailand. **Thai nat. Sci. Pap. Fauna. Ser., 2:** 1-35, 1968.

32. SHARPE, R.G.; HIMS, M.M.; HARBACH, R.E. & BUTLIN, R.K. - PCR-based methods for identification of species of the *Anopheles minimus* group: allele-specific amplification and single-strand conformation polymorphism. **Med. vet. Entomol., 13:** 265-273, 1999.

33. SIMMONS, J.S.; WHAYNE, T.F.; ANDERSON, G.W. & HORACK, H.M. - **Global epidemiology. A geography of disease and sanitation.** Philadephia, J.B. Lippincott, 1944. v.1, p. 504.

34. SOMBOON, P. & MORAKOTE, N. - Infectivity of gametocytes of *Plasmodium falciparum* and *Plasmodium vivax* after storage *in vitro*. **Ann. trop. Med. Parasit., 84:** 89-91, 1990.

35. SOMBOON, P.; MORAKOTE, N.; KOOTTATHEP, S. & TRISANAROM, U. - Detection of sporozoites of *Plasmodium vivax* and *Plasmodium falciparum* in mosquitoes by ELISA: false positivity associated with bovine and swine blood. **Trans. roy. Soc. trop. Med. Hyg., 87:** 322-324, 1993.

36. SOMBOON, P. & SUWONKERD, W. - Establishment of a stenogamous colony of *Anopheles minimus* species A. **Ann. trop. Med. Parasit., 91:** 673-676, 1997.

37. SOMBOON, P.; WALTON, C.; SHARPE, R.G. *et al*. - Evidence for a new sibling species of *Anopheles minimus* from the Ryukyu Archipelago, Japan. **J. Amer. Mosq. Contr. Ass., 17:** 98-113, 2001.

38. SUCHARIT, S.; KOMALAMISRA, N.; LEEMINGSAWAT, S.; APIWATHNASORN, C. & THONGRUNGKIAT, S. - Population genetic studies on the *Anopheles minimus* complex in Thailand. **Southeast Asian J. trop. Med. publ. Hlth, 19:** 717-723, 1988.

39. VEJJASASTRA, P. - Malaria survey at Tung Song, Nakhon Si Thamarat. Department of Health (Thailand). **Publ. Hlth Bull.,** (11), 1933. (in Thai).

40. VERHAVE, J.P.; LEEUWENBERG, A.D.; PONNUDURAI, T.; MEUWISSEN, J.H. & VAN DRUTEN, J.A. - The biotin-streptavidin system in a two-site ELISA for the detection of plasmodial sporozoite antigen in mosquitoes. **Parasite Immunol., 10:** 17-31, 1988.

41. WORLD HEALTH ORGANIZATION - **Wkly epidem. Rec., 32:** 265-272, 1999.

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