Biting patterns and host preference of *Anopheles epiroticus* in Chang Island, Trat Province, eastern Thailand

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ABSTRACT: A study of species diversity of *Anopheles* mosquitoes, biting patterns, and seasonal abundance of important mosquito vectors was conducted in two villages of Chang Island, Trat Province, in eastern Thailand, one located along the coast and the other in the low hills of the central interior of the island. Of 5,399 captured female anophelines, 70.25% belong to the subgenus *Cellia* and remaining specimens to the subgenus *Anopheles*. Five important putative malaria vectors were molecularly identified, including *Anopheles epiroticus, Anopheles dirus, Anopheles sawadwongporni, Anopheles maculatus,* and *Anopheles minimus. Anopheles epiroticus* was the most commonly collected species in the coastal site, whereas *An. dirus* was found to be most abundant in the forest-hill site. From both locations, a greater number of mosquitoes was collected during the dry season compared to the wet. *Anopheles epiroticus* showed greater exophagic and zoophilic behavior with the highest blood feeding densities occurring between 18:00 and 19:00. In contrast, *An. dirus* demonstrated an activity peak between midnight and 01:00. We conclude that *An. epiroticus* and *An. dirus*, in coastal and inland areas, respectively, appear to be the most epidemiologically important malaria vectors on Chang Island. As no studies of vector competency specific to Chang Island have been conducted, our conclusions that these two species play a primary role in malaria transmission are based on evidence from other localities in Thailand and mainland Southeast Asia. This information serves as a basis for designing improved vector control programs that target specific species, and if integrated with other interventions could result in the elimination of malaria transmission on the island. ***Journal of Vector Ecology* 39 (2): 361-371. 2014.**

Keyword Index: *Anopheles*, species diversity, malaria, Chang Island, Thailand.

INTRODUCTION

In Thailand, malaria is a significant cause of morbidity and remains prevalent and entrenched in the more remote forested and hilly areas, especially along the international borders with Cambodia and Myanmar where efficient malaria vectors are common and access to health care distant (Kongmee et al. 2012). Of the approximately 73 *Anopheles* species recognized in Thailand, member species in the Leucosphyrus Group, Maculatus Group and Minimus Subgroup, include five of the primary malaria vectors (Rattanarithikul et al. 2006). Nine species of mosquitoes have been incriminated as malaria vectors in Thailand (Green et al. 1991, Rattanarithikul et al. 2006, Suwonkerd et al. 2013), including *Anopheles dirus* (Baimai et al. 1988, Rosenburg et al. 1990), *An. baimaii* (Baimai et al. 1988, Green et al. 1991), *An. minimus* (Ratanatham et al. 1988, Rattanarithikul et al. 1996), *An. pseudowillmori*, *An. maculatus* (Cheong et al. 1968), *An. aconitus* (Maheswary et al. 1992), *An. sawadwongporni,* and *An. campestris* (Somboon et al. 1998, Coleman et al. 2002), all of which are associated with hilly forest environments and forest-fringe areas. The last malaria vector, *An. epiroticus,* occurs along mainland coastal areas and islands where this species predominantly utilizes brackish water habitats (Sumruayphol et al. 2010).

Anopheles sundaicus s.l. is regarded as the principal vector of malaria along many coastal areas in Southeast Asia (Adak et al. 2005, Alam et al. 2006, Dusfour et al. 2007a). The species complex is widely distributed from northeastern India, eastwards to southern Vietnam (south of the 11th parallel), and southwards to the Andaman and Nicobar Islands (India), Malaysia (peninsular and northern Borneo), and Indonesia (Java, Sumatra, Sulawesi, and Lesser Sunda islands) (Linton et al. 2001, Dusfour et al. 2004a). At least four sibling species are recognized in the complex, *An. epiroticus* (formerly *An. sundaicus* species A), *An. sundaicus* s.s., *An. sundaicus* species E, and *An. sundaicus* species D (Dusfour et al. 2007b, Alam et al. 2006). In Thailand, only *An. epiroticus* is regarded as present and is found along the coastal regions and islands of the eastern and southern regions (Scanlon et al. 1968, Sukowati et al. 1996, 1999, Linton et al. 2005, Rattanarithikul et al. 2006) and has been incriminated as a secondary malaria vector (Gould et al. 1966, Harinasuta et al. 1974, Chowanadisai et al. 1989).

Chang Island is located in the Gulf of Thailand, (Ko Chang District, Trat Province), eastern Thailand, 30 km east

of the mainland (Figure 1). Although Chang Island is a major tourist destination, it is also a malaria endemic area that poses a health risk to visitors and local inhabitants alike. The island is roughly divided into either forested foothills or coastline in which malaria remains a significant health threat and concern. From 2006 to 2013 a total of 201 malaria cases were recorded from the island. During this period, the number of infections peaked in 2006 with 61 cases including *Plasmodium falciparum* and 52 *P. vivax* infections (Bureau of Vector Borne Diseases 2013). While malaria is endemic, information is lacking not only on the diversity of mosquitoes present, but also medical importance (vector status), basic biology, and behavioral aspects of each. Vector incrimination and species bionomics is critical for defining the vectorial capacity of each species, understanding the spatial and temporal disease transmission risk, and for designing appropriate vector prevention and control strategies against target species.

Recently, molecular-based assays have been developed to reliably identify individual species within the different *Anopheles* complexes (Manguin et al. 2008). An allele-specific polymerase chain reaction assay using cytochrome oxidase I (COI) and cytochrome-*b* (Cyt-b) has been developed for distinguishing three allopatric species of the Sundaicus Complex that includes *An. epiroticus* (Thailand, Vietnam, Cambodia, peninsular Malaysia), *An. sundaicus* s.s (Borneo Malaysia, Kalimantan Indonesia), and *An. sundaicus* E (Sumatra, Java, Lesser Sunda Islands, Indonesia) (Dusfour et al. 2007a). Additionally, *An. epiroticus* can be identified using the internal transcribed spacer 2 (ITS2), domain 3 (D3), and COI (Sumruayphol et al. 2010). Accurate identification of sibling species of important vectors contributes to more accurate studies of vector species and effective control of target species (Sinka et al. 2011, Kongmee et al. 2012).

As there is very limited bionomic information about anophelines on the island, this study was designed to describe the trophic behavioral patterns (biting cycle and feeding preferences) and seasonal abundance of *An. epiroticus* and other species present on Chang Island.

MATERIALS AND METHODS

Study sites

Chang Island (Ko Chang) is located approximately 350 km from Bangkok in Trat Province, eastern Thailand, near the border of Cambodia. Chang Island is the second largest island in the Gulf of Thailand, covering an area of approximately 217 km^2 with a human population of nearly 8,000. Most of the island consists of forested foothills and coastal zones mostly covered by tropical rainforest.

Anopheles mosquitoes were collected from two different sites on the island, including a coastal site at Khlong Yuan Village (12° 02'N, 102° 23'E) and an inland forest site at Khlong Jao Lueam waterfall (12° 06'N, 102° 18'E) (Figure 1). Coastal Khlong Yuan Village is 500 m from the sea at an elevation of approximately 39 m above sea level and is surrounded by fruit orchards and rubber plantations along with natural mangrove forests. Interior Khlong Jao Lueam is near a waterfall surrounded by deep forests and high hills. Khlong Jao Lueam site is approximately 2.5 km from the sea and approximately 71 m above sea level.

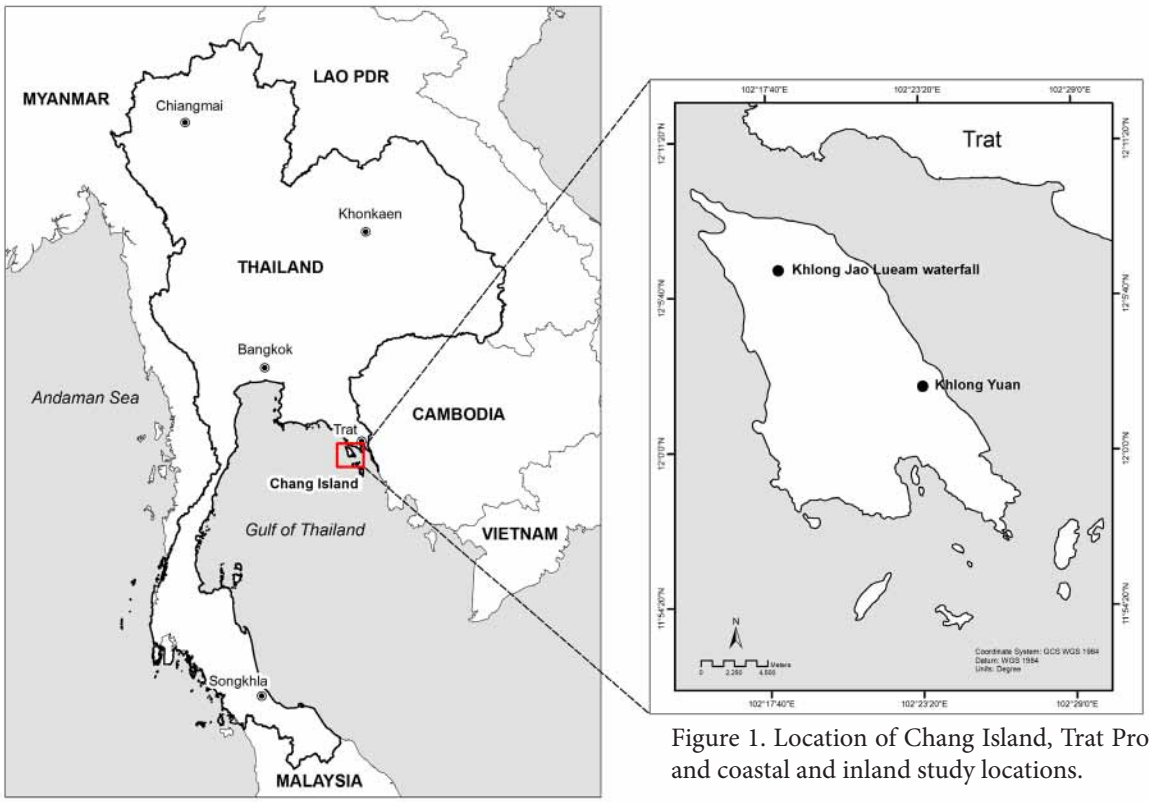

Figure 1. Location of Chang Island, Trat Province, Thailand, and coastal and inland study locations.

Collection methods

Adult mosquitoes were collected once every two months during three consecutive nights for a period of two years (2011 to 2012). Mosquito collections were carried out using up to three methods: human-landing indoor (HLI), human-landing outdoor (HLO), and buffalo-bait collections (BBC) when applicable. Human-landing collections (HLC) were performed in a local (inhabited) house in each site. Each site consisted of two mosquito collection teams (four persons each), with each team divided into two groups of two collectors each for indoor and outdoor activities, respectively. Mosquitoes were collected from 18:00 to 06:00 with each team dividing collection time from the first half of the night (18:00 to 24:00), followed by the second team beginning at midnight to 06:00. Every hour the team members rotated between indoor and outdoor collection positions to mitigate collector bias. Outdoor collectors sat within 50 m of the study house. HLC were done for 45 min each hour (e.g., 18:00-18:45). Each collector captured mosquitoes landing on exposed legs (knee to foot) using a flashlight and a mouth aspirator. Approval for the HLC activity was provided by the Ethics Review Committee for Research Involving Human Subjects, Health Science Group of Faculties, Colleges and Institutes, Chulalongkorn University, Thailand (Approval COA No. 167/2013).

The buffalo-baited capture (BBC) method was only performed in Khlong Yuan Village as the animal represented the only buffalo (*Bubalus bubalis*) on the entire island, The method placed a tethered animal inside a suspended net 20 m^2 in area (2 m height) in a designated holding area to attract host-seeking mosquitoes. Netting was set up from ground level with a small gap (0.3 m) to allow the mosquitoes access. Mosquitoes that entered were allowed to rest or blood feed and subsequently rest on the inside of the netting before collection using a mouth aspirator. The BBC was performed 15 min each hour from 18:00-06:00. *Anopheles* females were held in plastic cups, labeled by hour and site of collection, and covered with netting and a cotton pad saturated with 10% sugar solution. Mosquitoes were transferred to another location for morphological identification and sorting. Ambient air temperature and relative humidity were recorded from indoor and outdoor locations each hour. At the site of the buffalo-baited trap, a manual thermo-hygrometer (BARICO GmbH, Villingen-Schwenningen, Germany) was used to collect the hourly air temperature and percent relative humidity. Rainfall data was obtained from the Trat Province meteorological station located approximately 110 km from the study sites.

Morphological identification

Anopheles females were initially identified and sorted in the field using a standard morphological identification key (Rattanarithikul et al. 2006). Afterward, all specimens were preserved in liquid nitrogen and returned to Kasetsart University, Bangkok, for molecular identification and further analysis.

Molecular identification

DNA from specimens initially identified as *An. sundaicus* s.l., *An. minimus* s.l., *An. dirus* s.l., and *An. maculatus* s.l. were extracted from each adult mosquito following the methods of Linton et al. (2001) and Manguin et al. (2002). Molecular identification of members of the *An. sundaicus* complex used the AS-PCR assay developed by Dusfour et al. (2007a) using species-specific primers for *An. sundaicus* s.s, *An. epiroticus,* and *An. sundaicus* E. For the Dirus Complex, the AS-PCR method of Walton et al. (1999) was performed using specific primers for *An. dirus*, *An. cracens*, *An. scanloni*, *An. baimaii,* and *An. nemophilous.* For members of *An. minimus* complex, the AS-PCR assay of Garros et al. (2004) was conducted using the specific primers for *An. minimus* and *An. harrisoni*. For the *An. maculatus* group, molecular identifications were performed using the AS-PCR assay of Walton et al. (2007) using specific primers for *An. maculatus* and *An. sawadwongporni*.

Data analysis

Seasons, collection times, locations, and specific collection methods were used in the analysis. Seasonal periods were separated to include wet (May to October) and dry (November to April) seasons, collection time periods were group classified as early evening (18:00–21:00), late night (21:00–24:00), predawn (24:00–03:00), and dawn (03:00–06:00), and collection types and locations were designated as HLI, HLO, and BBC. The nocturnal biting behavior of *An. epiroticus* was tabulated by averaging the number of *Anopheles* landing per hour per human, separated by indoor and outdoor positions and by averaging the number of mosquitoes captured in the buffalo trap for 15 min each hour. Comparisons of human-landing data were analyzed by non-parametric Kruskal-Wallis, Wilcoxon, and Mann-Whitney tests. The level of significance was set at 0.05% (P valve < 0.05), followed by correlation coefficient (r) analysis taking into account specimens captured and environmental variables. All data were analyzed using the SPSS statistical package (SPSS version 17.0, Chicago, IL).

RESULTS

In the two-year period, combined study sites had a total of 5,399 anophelines captured, comprising 14 *Anopheles* taxa separated within two subgenera, *Cellia* and *Anopheles* (Table 1), representing nine (n=3,793, 70.25%) and five (n= 1,606), respectively. From all collections, 97.5% (5,264) were captured from the coastal site of Klong Yuan and only 2.5% (135) were collected from the inland forested location. However, buffalo-baited trapping only occurred in Khlong Yuan and represented 83.2% of all anophelines collected. Excluding the BBC data, when comparing only HLC data, Khlong Yuan still produced the majority (85.1%) of captured anophelines compared to Khlong Jao Lueam. Within the subgenus *Cellia,* 3,444 specimens (90.8%) belonged to the Sundaicus Complex, 140 (3.7%) within the Dirus Complex, 43 (1.13%) to the Maculatus Group, and nine (0.24%) to the Minimus Complex. Many members within these four taxonomic assemblages are

regarded as potential malaria vectors in Thailand (Saeung 2012). Species that are typically regarded as non-malaria vectors within the subgenus included *Anopheles jamesii*, *An. kochi*, *An. karwari*, *An. vagus,* and *An. philippinensis* (Table 1). Additionally, five taxa within the subgenus *Anopheles* were identified, including *An. umbrosus*, *An. barbirostris* group, *An. aitkenii* group, *An. hyrcanus* group, and *An. peditaeniatus* (Table 1). Only members in the *Barbirostris* and *Hyrcanus* groups have been found infected with either *P. falciparum* and/or *P. vivax* parasites (Rattanarithikul et al. 1996).

Only specimens from the four putative malaria vector species complexes or groups in the subgenus *Cellia* were subjected to definitive species identification using the appropriate multiplex AS-PCR assay. Five important species were identified, including *An. dirus* (former *An. dirus* species A) (3.9% of samples assayed), *An. minimus* (former *An. minimus* species A) (0.25%), *An. sawadwongporni* (0.8%), *An. maculatus* (0.4%), and *An. epiroticus* (former *An. sundaicus* species A) (94.7%). The initial morphological identification recorded 14 species or assemblages, whereas follow-up PCR allowed the identification of *An. sawadwongporni* (Maculatus Group), resulting in a total of 15 species collected on Chang Island. The multiplex assay was able to identify nearly 90% of all assayed specimens initially identified as members of the Sundaicus Complex, whereas the remaining 10% were not identifiable by PCR for reasons that remain unclear (Figure 2). However, following the most current revision of the Sundaicus Complex in Southeast Asia, *An. epiroticus* is the only species believed present in Thailand (Suwonkerd et al. 2013).

In Klong Yuan, *An. epiroticus* was the most abundant (90.8%) of the 15 *Anopheles* species identified and when compared to only the other four key potential malaria vectors present, it contributed to 98.4% of the collection (Tables 1 and 2). *Anopheles epiroticus* was not collected from Khlong Jao Lueam. At this latter site, only two species were identified among 135 *Anopheles* captured, the majority being *An. dirus* (94.1%) followed by *An. minimus* (5.9%) (Table 2).

The majority of *An. epiroticus* from Klong Yuan were captured in the buffalo trap (78.4%) compared to HLC. The distribution of this species in the HLC found 65.5% outdoors compared to timed-matched indoor collections (Table 3). With 21.6% of *An. epiroticus* collected on humans either indoors or outdoors, this species demonstrated some degree of anthropophily but appears attracted to both humans and buffalo (alternative animal blood sources). The *An. epiroticus* feeding patterns by hour and collection method are shown in Figures 3A and 3B. The indoor human biting activity showed small peaks from 01:00-02:00 and 03:00-05:00. The outdoor human biting activity presented two small peaks from 21:00-22:00 and 01:00-02:00. None of these small increases in biting density are considered marked and it appears this species is active throughout the evening, both indoors and out (Figure 3A). In contrast, the mosquito activity patterns associated with buffalo bait showed the greatest activity at the beginning of the collection period (18:00 to 19:00), and declining progressively thereafter until the following morning (Figure 3B).

A greater number of *An. epiroticus* were collected during the dry season (November to April) with a notable peak

Table 1. Total numbers of *Anopheles* mosquitoes collected at Chang Island, Trat Province, from January, 2011 to September, 2012 based on initial morphological species identification.

Anopheles species	Khlong Yuan		Khlong Jao Lueam	Total
	Human bait	Buffalo bait	Human bait	
Anopheles (Cellia)				
An. dirus s.l.	5	8	127	140
An. minimus s.l.	1	0	8	9
An. maculatus s.l.	0	43	0	43
An. sundaicus s.l.	743	2,701	0	3,444
An. jamesii	0	25	0	25
An. kochi	6	90	0	96
An. karwari	1	32	0	33
An. vagus	0	1	0	1
An. philippinensis	0	2	0	2
Anopheles (Anopheles)				
An. umbrosus	14	382	0	396
An. barbirostris group	0	12	0	12
An. aitkenii group	0	175	0	175
An. hyrcanus group	0	997	0	997
An. peditaeniatus	0	26	0	26
Total	770	4,494	135	5,399

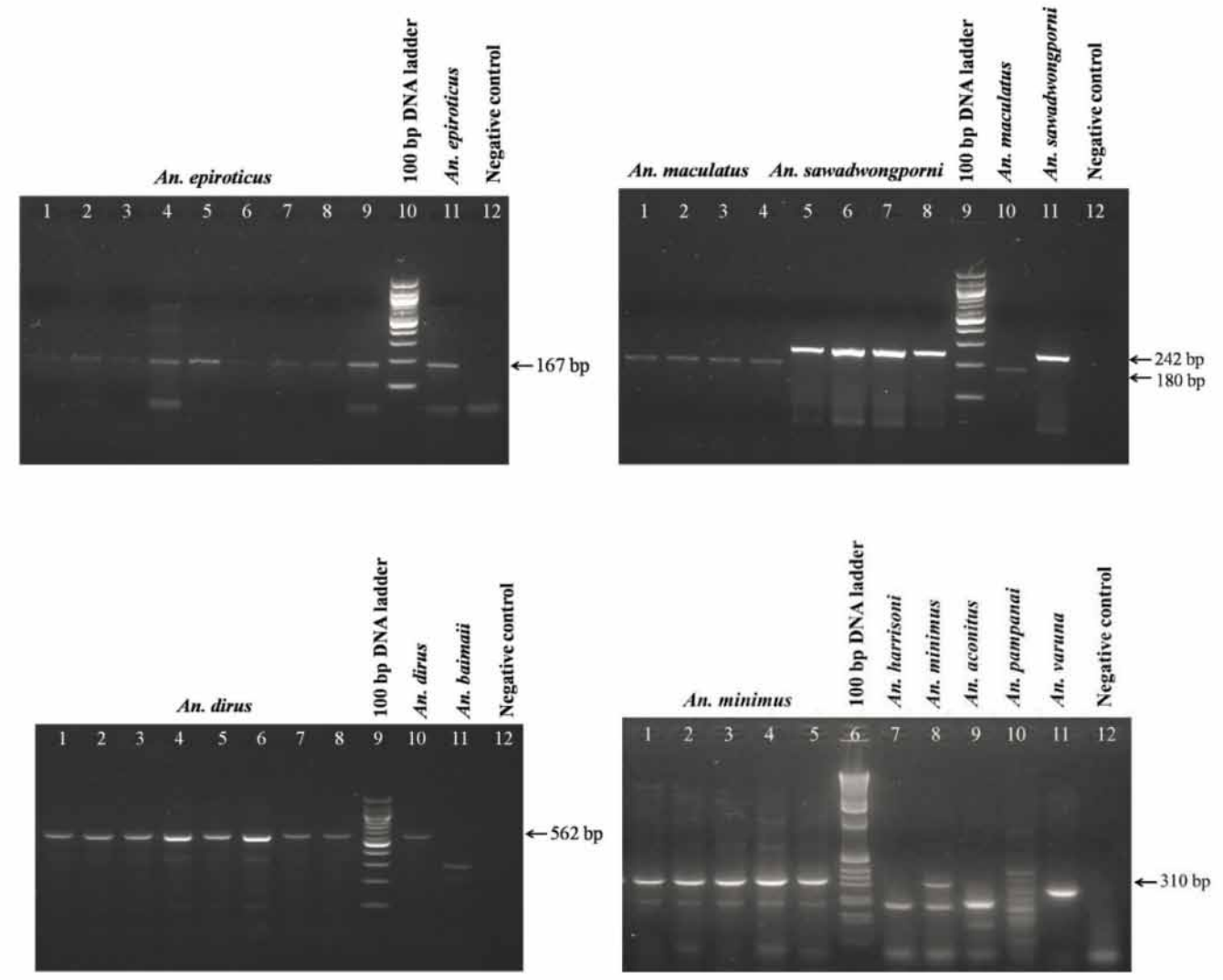

Figure 2. Amplified fragments using allele-specific PCR assay for identifying *An. epiroticus* *An. maculatus*, *An. sawadwongporni*, *An. dirus,* and *An. minimus* mosquitoes with appropriate control sample DNA.

in the early dry season from November to January (Figure 4). In contrast, *An. epiroticus* was found in relatively low adult densities during the wet season (May to October). In the inland forest site, both *An. dirus* and *An. minimus* were collected in higher abundance during the dry season (peak January-March).

Comparisons of HLC data were analyzed by non-parametric Kruskal-Wallis, Wilcoxon, and Mann-Whitney tests. A strong significant difference in the number of *An. epiroticus* was found between seasons (Z= -4.696, $P = 0.000$), and between indoor, outdoor human landing, and buffalo-baited collection methods (F = 5.319, df=2, $P = 0.010$). The Wilcoxon pairwise comparison between indoor vs outdoor collections (Z = -2.803, $P = 0.005$), between indoor vs buffalo (Z = -2.936, $P = 0.003$), and between outdoor vs buffalo (Z = -2.994, $P = 0.003$) were significantly different from one another. There was no significant difference in the number of *An. epiroticus* collected between the four quarterly evening time intervals ($\chi^2=0.04$, $df=3$, $P=0.998$ indoor; $\chi^2=0.91$, $df=3$, $P=0.823$ outdoor and $\chi^2=0.579$, $df=3$, $P=0.903$ buffalo). Data from all collection methods were pooled to determine the correlation between mosquito abundance and measured environmental variables (Table 3 and Figures 3A, 3B). Results indicated that *An. epiroticus* densities were strongly correlated with rainfall patterns ($r = -0.667$; $P = 0.009$) and relative humidity ($r = -0.640$; $P = 0.012$) but were not associated with relative minimum or maximum ambient air temperatures ($P > 0.05$).

DISCUSSION

In this study, five important malaria vectors in Thailand were molecularly identified as occurring on Chang Island, including *An. dirus*, *An. minimus, An. maculatus, An. sawadwongporni,* and *An. epiroticus*. *Anopheles epiroticus* was only found at Khlong Yuan, a coastal site. In contrast, *An. dirus*, arguably the most efficient malaria vector in Thailand, was identified in Khlong Jao Lueam, an inland site near forested hills. Khlong Yuan contained all five key vector species and showed much higher anopheline species diversity (15 species in all) compared to only two species captured in Khlong Jao Lueam, of which *An. dirus* represented 94% of the catch. Khlong Yuan contains several potential breeding habitats for *An. epiroticus*, a species that typically requires/prefers sunlit, mostly brackish, water habitats containing floating algae (Sinka et al. 2011). The most favorable habitats are abandoned or poorly maintained shrimp/fish ponds or inland seawater canals, but immature stages will also inhabit coastal ponds, swamps, mangrove, and rock pools (Manguin et al. 2008). In our study, we attempted sampling of all available potential aquatic habitats in and around each study site every two months during the same time as adult collections; however, larval collections were generally unproductive and not particularly informative. We were unable to identify any productive larval habitats of *An. epiroticus* in and around Khlong Yuan. The inability to detect immature stages of this species is puzzling and something that was entirely unexpected given its relatively high abundance in adult collections. A careful, systematic search for all possible larval habitats to include greater geographical coverage along the coastline of Chang Island is required.

The behavior of *An. sundaicus* sibling species appears to differ depending on the locality (Dusfour et al. 2004a, Linton et al. 2005). The biting activity of *An. sundaicus* complex typically occurs between 20:00 to 03:00 depending on the locality (Sinka et al. 2011). In Thailand, Gould et al. (1966) observed that *An. sundaicus* s.l. (= *An. epiroticus*) had a greater outdoor biting frequency and feeding preference on cows, indicating more pronounced exophagy and zoophily. In contrast, the trophic behavior of *An. sundaicus* s.l. in Cambodia varied from exophagy to endophagy (Dusfour et al. 2004a). In Rayong Province, Thailand, Sumruayphol et al. (2010) observed *An. epiroticus* blood feeding predominantly between 18:00-24:00 with a peak of biting activity around midnight with a maximum of 6.6 bites/person/h. In Chang Island, we found the buffalo to be a potent attractant for *An. epiroticus* relative to humans, indicating stronger zoophilic behavior. Buffalo-baited collections showed the highest activity during the beginning of the 12-h collection period between 18:00 and 19:00. Although we recognize that the movement of *An. epiroticus* to bait and blood feeding could have taken place before 18:00, it would have unlikely occurred in high numbers outside forested or well-shaded environments. The biting patterns of *An. epiroticus* in indoor and outdoor human landing collections were very similar, and only small peaks of activity were observed between 01:00-02:00 indoors and between 20:00-21:00 and 01:00-02:00 in outdoor captures. The limited number of specimens of *An. dirus* (140), *An. minimus* (9), *An. maculatus* (15), and *An. sawadwongporni* (28) collected in this study did not allow for accurate interpretations of host-seeking patterns or definitive conclusions regarding seasonality and species abundance.

The seasonal abundance of *An. epiroticus* in this study appeared to be influenced by several factors, most notably precipitation patterns. The population densities of *An. epiroticus* showed the greatest abundance during the dry season (November to April) for both the first and second years of observations. *An. epiroticus* was found active every collection period throughout the two years with the highest densities in November. In Indonesia, high densities of *An. sundaicus* s.l. were also associated with the dry season as lagoons and brackish water impoundments became more suitable habitats as water flow was impeded from entering the sea, creating large stagnant bodies of water and abundant floating algal mats (Sundararaman et al. 1957). However, in Rayong Province, *An. epiroticus* was found active throughout the year with the highest densities in the rainy season (September). We also noted a significant negative association with adult densities and with higher mean ambient relative humidity.

Thailand faces recurring threats of emerging and re-emerging arthropod-borne diseases, especially malaria (Chareonviriyaphap et al. 2013). Malaria remains most prevalent along the less developed international borders of eastern Myanmar, northern Malaysia, and western Cambodia, as well as several coastal zones where the blackish water

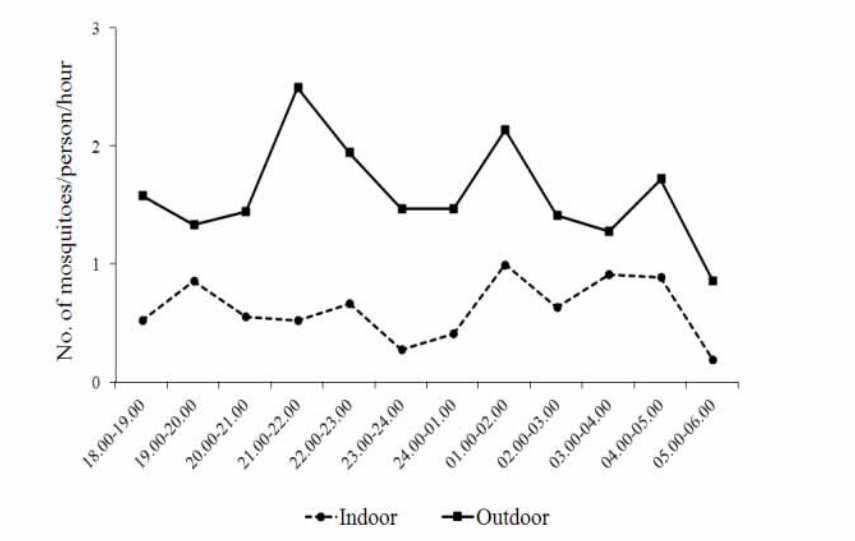

Figure 3A. Mean hourly densities of *Anopheles epiroticus* by human (indoor and outdoor) landing collections in Khlong Yuan.

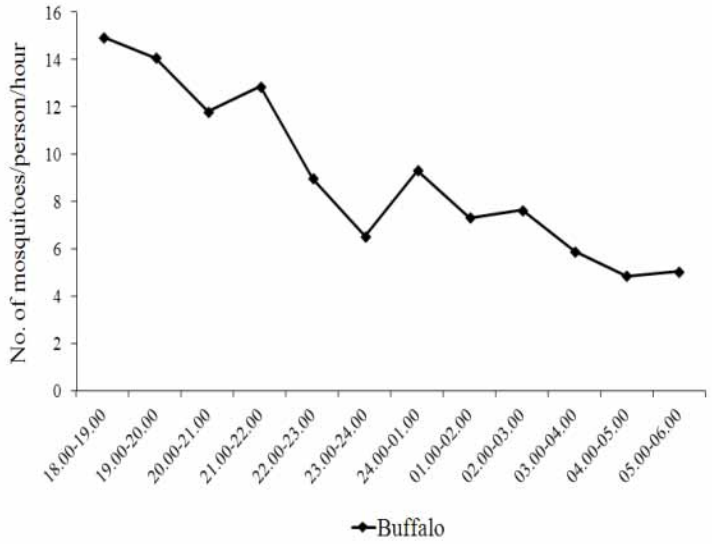

Figure 3B. Mean hourly densities of *Anopheles epiroticus* using a buffalo-baited trap in Khlong Yuan.

mosquito, *An. epiroticus*, exists. Chang Island is the second largest island of Thailand and is regarded as one of the most attractive and popular tourist sites in the country. However, this island is still a malaria endemic area with around 50-100 cases annually since the beginning of the 1990s. However, in 2013, only five malaria cases (all *P. vivax*) were detected and presumably transmitted on the island. The malaria risk areas are generally located in two relatively undeveloped areas on the island, including a forest fringe in the interior hills and a coastal area where *An. dirus* and *An. epiroticus* are present, respectively. The Chang National Park on the eastern side of the island is primarily an inland forest with creeks, rivers, and waterfalls which provide many potential habitats for malaria vectors.

Although malaria vectors have been identified on Chang Island, no information about their biology and behavior (population dynamics, biting and host preference, and seasonal abundance) have been described from the island. Moreover, until this study, there had been no attempt to identify the species present based on molecular methods. A critical component to understanding the local epidemiology is a precise identification of the vector species in various locales. The vectorial capacity of different sibling species can often vary in behavior, resulting in different abilities to transmit malaria. Such information is important to help identify the respective roles of each vector species in disease transmission and to implement appropriate prevention and control strategies.

Anopheles epiroticus belongs to the Sundaicus Complex in the Pyretophorus Series, a grouping of important malaria

Table 2. Numbers of select *Anopheles* species (as suspected malaria vectors) based on molecular analysis collected at Chang Island, Trat Province, between January, 2011 and September, 2012.

Anopheles	Khlong Yuan			Khlong Jao Lueam		Total
	Buffalo	Indoor	Outdoor	Indoor	Outdoor	
An. dirus	8	0	5	58	69	140
An. minimus	0	0	1	8	0	9
An. sawadwongporni	28	0	0	0	0	28
An. maculatus	15	0	0	0	0	15
*An. epiroticus**	2,701	256	487	0	0	3,444
Total	2,752	256	493	66	69	3,636

*Approximately 10% of samples failed to generate a PCR-based confirmation, however, based on initial morphological identification, all specimens were deemed *An. epiroticus*.

Table 3. Total of monthly captures of *An. epiroticus* from three collection methods in Chang Island, Trat Province.

Month	In	Out	Buffalo	Total	Mean		
					Temp. (°C)	RH (%)	Rainfall (mm)
Year 1							
Jan 11	40	52	310	402	26.8	65	0.0
Mar 11	29	33	395	457	27.0	78	235.6
May 11	18	27	45	90	28.4	83	353.5
Jul 11	1	0	2	3	27.1	86	895.8
Sep 11	0	0	3	3	26.6	88	1446.7
Nov 11	77	179	583	839	28.2	73	9.5
Year 2							
Jan 12	76	119	716	911	27.3	76	141.1
Mar 12	8	28	32	68	28.1	80	136.5
May 12	1	3	10	14	27.6	86	622.9
Jul 12	0	1	0	1	27.0	87	857.4
Sep 12	0	2	3	5	26.3	89	1311.0
Nov 12	6	43	602	651	27.7	84	392.8
Total	256	487	2,701	3,444			

RH = percent relative humidity.

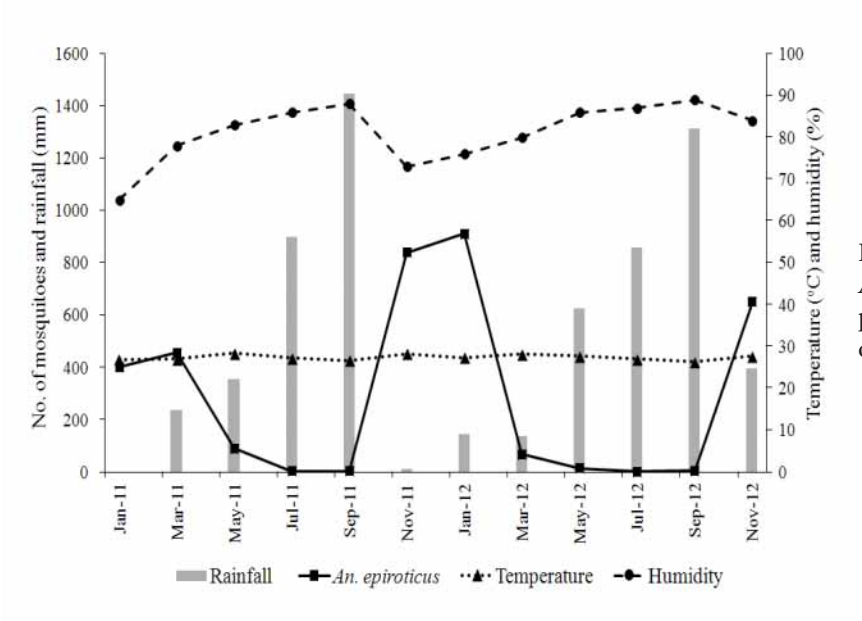

Figure 4. Seasonal abundance of *An. epiroticus* in relation to monthly precipitation and percent relative humidity on Chang Island, Trat Province.

vectors in Asia and Africa (Harbach 2013). Members of this species complex have long been recognized as malaria vectors in coastal areas and on islands in Southeast Asia (Sukowati et al. 1999, Dusfour et al. 2004b, Linton et al. 2005); however, their relative importance as either major or secondary malaria vectors varies by locality and epidemiological factors influencing transmission (Schaefer and Kirnowardayo 1983, Dusfour et al. 2007a). Of the four known allopatric species in the complex, only *An. epiroticus* has been identified in Thailand and distributed in eastern and southern coastal areas (Rattanarithikul et al. 2006, Dusfour et al. 2004b, Linton et al. 2005, Dusfour et al. 2007b). With the development of PCR assays that identify individual sibling species, a growing number of studies have successfully described the trophic behavior, biting activity, and seasonal abundance of several mainland malaria vector species in Thailand (Chareonviriyaphap et al. 2003, Sungvornyothin et al. 2006, Muenworn et al. 2008, Tananchai et al. 2012, Tisgratog et al. 2012, Kongmee et al. 2012), whereas similar investigations on coastal and island species, like *An. epiroticus*, has been limited.

Although *An. epiroticus* is typically regarded as a secondary vector of malaria (Harinasuta et al. 1974), its potential as an efficient vector remains a prime concern near tourist areas and local coastal settlements on Chang Island. The information gathered in this study indicates two primary vectors on the island: *An. epiroticus* along the coastal zone and *An. dirus* in the interior parts. Although malaria parasites were not tested in any of the anophelines captured on humans or in buffalo-baited traps, these two species were the predominant vectors in their respective localities throughout much of the study period, albeit adult densities varied by season. Therefore, from an epidemiological perspective, all available evidence implicates both anophelines as the most likely vector candidates. As only two sites were observed, this does not preclude other potential vector species playing a greater role in transmission on the island. Further investigations in other localities will better define and map vector distribution in relation to malaria transmission. Moreover, a far better understanding of the full range and preferred *An. epiroticus* larval habitats is also required and a pre-requisite to any meaningful attempt to control this species and transmission of malaria.

Recent malaria statistics reveal that malaria transmission has been lower than in the past, especially since 2006 when a high of 113 cases was reported. Between 2007 and 2013, an average of less than 13 cases of malaria per year occurred. For this reason, it may be feasible to attempt an island-wide integrated campaign to eliminate malaria entirely from the island by identifying all residual foci and treating all human reservoirs of malaria. For those areas with evidence of recent or high risk for renewed transmission, a time-limited vector control strategy could also complement the elimination effort when appropriate. As importation of malaria into Chang Island remains a threat, any successful elimination effort will still require a robust surveillance system to be in place to quickly identify cases and prevent secondary transmission. To advance the most appropriate vector control on Chang Island, additional investigations are needed on vector biology and transmission potential of local anopheline populations.

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REFERENCES CITED

Adak, T., O.P. Singh, M.K. Das, S. Wattal, and N. Nanda, 2005. Comparative susceptibility of three important malaria vectors *Anopheles stephensi*, *Anopheles fluviatilis*, and *Anopheles sundaicus* to *Plasmodium vivax*. J. Parasitol. 91: 79–82.

Alam, M.T., M.K. Das, M.A. Ansari, and Y.D. Sharma. 2006. Molecular identification of *Anopheles (Cellia) sundaicus* from the Andaman and Nicobar islands of India. Acta Trop. 97: 10–18.

Baimai, V., U. Kijehalao, P. Sawadwongporn, and C. A. Green. 1988. Geographic distribution and biting behaviour of four species of the *Anopheles dirus* complex (Diptera: Culicidae) in Thailand. Southeast Asian. J. Trop. Med. Publ. Hlth. 19: 151–161.

Bureau of Vector Borne Diseases. 2013. Database of Malaria. : http://203.157.41.98/malaria2013/freereoprt/rep1a.php, Accessed December 15, 2013.

Chareonviriyaphap, T., A. Prabaripai, M.J. Bangs, and B. Aum-Aung. 2003. Seasonal abundance and blood feeding activity of *Anopheles minimus* Theobald (Diptera: Culicidae) in Thailand. J. Med. Entomol. 40: 876-881.

Chareonviriyaphap, T., M. J. Bangs, W. Suwonkerd, M. Kongmee, V. Corbel, and R. Ngoen-Klan. 2013. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. Parasites Vectors 6: 280.

Cheong W.H., H.J. Fredericks, A.H. Omar, and F.L. Sta Maria. 1968. The incrimination of *Anopheles maculatus* as the vector of human malaria at the Kelantan-Thai border. Med. J. Malaya 22: 245.

Chowanadisai, L., S. Krairiksh, and S. Thanasripukdikul. 1989. Microbial control of *Anopheles sundaicus*, a malaria vector in Thailand, by *Bacillus sphaericus* 2362. Mosq. Borne Dis. Bull. 6: 39-44.

Coleman, R.E., R. Sithiprasasna, P. Kankaew, C. Kiaattiu, S. Ratanawong, B. Khuntirat, and J. Sattabongkot. 2002. Naturally occurring mixed infection of *Plasmodium vivax* VK210 and *P. vivax* VK247 in *Anopheles minimus* and *An. maculatus* (Diptera: Culicidae) in western Thailand. J. Med. Entomol. 39: 556-559.

Dusfour, I., R.E. Harbach, and S. Manguin. 2004a. Bionomics and systematics of the oriental *Anopheles sundaicus* complex in relation to malaria transmission and vector control. Am. J. Trop. Med. Hyg. 71: 518–524.

Dusfour, I., Y.M. Linton, A. Cohuet, R.E. Harbach, V. Baimai, H.D. Trung, M.S. Chang, A. Matusop, and S. Manguin. 2004b. Molecular evidence of speciation between island and continental populations of *Anopheles* (*Cellia*) *sundaicus* Rodenwaldt (Diptera: Culicidae), a principal malaria vector in Southeast Asia. J. Med. Entomol. 41: 287–295.

Dusfour, I., J. Blondeau, R.E. Harbach, I. Vythilingham, V. Baimai, H.D. Trung, T. Sochanta, M.J. Bangs, and S. Manguin. 2007a. Polymerase chain reaction identification of three members of the *Anopheles sundaicus* (Diptera: Culicidae) complex, malaria vectors in Southeast Asia. J. Med. Entomol. 44: 723–731.

Dusfour, I., J.R. Michaux, R.E. Harbach, and S. Manguin. 2007b. Speciation and phylogeography of the Southeast Asian *Anopheles sundaicus* complex. Infect. Genet. Evol. 7: 484–493.

Garros, C., L.L. Koekemoer, M, Coetzee, M. Coosemans, and S. Manguin. 2004. A single multiplex assay to identify major malaria vectors within the African *Anopheles funestus* and the Oriental *Anopheles minimus* groups. Am. J. Trop. Med. Hyg. 70: 583-590.

Gould, D.J., J.E. Scanlon, M.S.C. Major, and R.A. Ward. 1966. *Anopheles* vectors of malaria in Southeast Asia. Washington, DC: Army Science Conference Proceedings. pp. 361-373.

Green, C.A., R. Rattanarithikul, S. Pongparit, P. Sawadwongporn, and V. Baimai. 1991. A newly-recognized vector of human malarial parasites in the Oriental region, *Anopheles* (*Cellia*) *pseudowillmori* (Theobald, 1910). Trans. R. Soc. Trop. Med. Hyg. 85: 35–36.

Harbach, R.E. 2013. The phylogeny and classification of *Anopheles*. In: S. Manguin (ed.). Anopheles *Mosquitoes - New Insights into Malaria Vectors*. InTech Open Access Publisher, Rijeka, Croatia. http://dx.doi.org: 10.5772/56117.

Harinasuta, C., P. Guptavanij, and C. Vasuvat. 1974. Studies on the medical ecological epidemiology in mangrove areas in Thailand. Southeast Asian J. Trop. Med. Pub. Hlth. 5: 105–127.

Kongmee, M., N.L. Achee, K. Lerdthusnee, M.J. Bangs, S. Chowpongpang, A. Prabaripai, and T. Chareonviriyaphap. 2012. Seasonal abundance and distribution of *Anopheles* larvae in a riparian malaria endemic area of western Thailand. Southeast Asian J. Trop. Med. Publ. Hlth. 43: 601-613.

Linton, Y.M., R.E. Harbach, S. Chang Moh, T.G. Anthony, and A. Matusop. 2001. Morphological and molecular identity of *Anopheles (Cellia) sundaicus* (Diptera: Culicidae), the nominotypical member of a malaria vector species complex in Southeast Asia. Syst. Entomol. 26: 357–366.

Linton, Y.M., I. Dusfour, T.M. Howard, L.F. Ruiz, Nguyen Duc Manh, Trung Ho Dinh, T. Sochanta, M. Coosemans, and R.E. Harbach. 2005. *Anopheles* (*Cellia*) *epiroticus* (Diptera: Culicidae), a new malaria vector in Southeast Asian Sundaicus complex. Bull. Entomol. Res. 95: 329-339.

Maheswary, N.P., M.A. Habib, and M. Elias. 1992. Incrimination of *Anopheles aconitus* Donitz as a vector of epidemic malaria in Bangladesh. Southeast Asian J. Trop. Med. Publ. Hlth. 13: 798-801.

Manguin, S., P. Kengne, L. Sonnier, R.E. Harbach, V. Baimai, H.D. Trung, and M. Coosemans. 2002. SCAR markers and multiplex PCR-based identification of isomorphic species in the *Anopheles dirus* complex in Southeast Asia. Med. Vet. Entomol. 16: 46–54.

Manguin, S., C. Garros, I. Dusfour, R.E. Harbach, and M. Coosemans. 2008. Bionomics, taxonomy, and distribution of the major malaria vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review.

Infect. Genet. Evol. 8: 489–503.

Muenworn, V., S. Sungvornyothin, K. Kongmee, S. Polsomboon, M.J. Bangs, P. Akrathanakul, S. Tanasinchayakul, A. Prabaripai, and T. Chareonviriyaphap. 2008. Biting activity and host preference of the malaria vectors *Anopheles maculatus* and *Anopheles sawadwongporni* (Diptera: Culicidae) in Thailand. J. Vector Ecol. 34: 62–69.

Ratanatham, S., E.S. Upatham, C. Prasittisuk, W. Rojanasunan, N. Theerasilp, A. Tremongkol, and V. Viyanant. 1988. Bionomics of *Anopheles minimus* and its role in malaria transmission in Thailand. Southeast Asian J. Trop. Med. Publ. Hlth. 19: 283–289.

Rattanarithikul, R., E. Konishi, and K.J. Linthicum. 1996. Detection of *Plasmodium vivax* and *Plasmodium falciparum* circumsporozoite antigen in anopheline mosquitoes collected in southern Thailand. Am. J. Trop. Med. 54: 114-121.

Rattanarithikul, R., B.A. Harrison, R.E. Harbach, P. Panthusiri, and R.E. Coleman. 2006. Illustrated keys to the mosquitoes of Thailand. IV. *Anopheles*. Southeast Asian J. Trop. Med. Publ. Hlth. 37 (Suppl.2): 1–128.

Rosenberg, R., R.G. Andre, and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 89: 22-28.

Saeung, T. 2012. *Anopheles* (Diptera: Culicidae) species complex in Thailand: Identification, distribution, bionomics and malaria-vector importance. Int. J. Parasitol. Res. 4: 75-82.

Scanlon, J.E., E.L. Peyton, and D.J. Gould. 1968. An annotated checklist of the *Anopheles* of Thailand. Thai Natl. Sci. Pap. Fauna Ser. 2: 1-35.

Schaefer, C. H. and S. Kirnowardoyo. 1983. An operational evaluation of *Bacillus thuringiensis* serotype H-14 against *Anopheles sundaicus* in West Java, Indonesia. Mosq. News 43: 325-328.

Sinka, M.E., M.J. Bangs, S. Manguin, T. Chareonviriyaphap, A.P. Patil, W.H. Temperley, P.W. Gething, I.R.F. Elyazar, C.W Kabaria, R.E. Harbach, and S.I. Hay. 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasites Vectors 4: 89.

Somboon, P., A. Aramrattana, J. Lines, and R. Webber. 1998. Entomological and epidemiological investigations of malaria transmission in relation to population movements in forest areas of north-west Thailand. Southeast Asian J. Trop. Med. Publ. Hlth. 29: 3-9.

Sukowati, S., V. Baimai, and H. Andris. 1996. Sex chromosome variation in natural populations of the *Anopheles sundaicus* complex from Thailand and Indonesia. Mosq. Borne Dis. Bull. 13: 8-13.

Sukowati, S., V. Baimai, S. Harun, Y. Dasuki, H. Andris, and M. Efriwati. 1999. Isozyme evidence for three sibling species in the *Anopheles sundaicus* complex from Indonesia. Med. Vet. Entomol. 13: 408-414.

Sumruayphol, S., C., Apiwathnasorn, N. Komalamisra, J. Ruangsittichai, Y. Samung, and P. Chavalitshewinkoon-Petmitr. 2010. Bionomic status of *Anopheles epiroticus* Linton & Harbach, a coastal malaria vector, in Rayong Province, Thailand. Southeast Asian J. Trop. Med. Pub. Hlth. 41: 541-547.

Sundararaman, S., R. M. Soeroto, and M. Siran. 1957. Vectors of malaria in Mid-Java, Indian J. Malar. 11: 321-338.

Sungvornyothin, S., V. Muenvorn, C. Garros, A. Prabaripai, M.J. Bangs, S. Manguin, and T. Chareonviriyaphap. 2006. Trophic behavior and biting activity of the two sibling species of the *Anopheles minimus* Complex in western Thailand. J. Vector Ecol. 31: 252–261.

Suwonkerd, W., W. Ritthison, C.T. Ngo, K. Tainchum, M.J. Bangs, and T. Chareonviriyaphap. 2013. Vector biology and malaria transmission in Southeast Asia. In: S. Manguin (ed.). Anopheles *Mosquitoes - New Insights into Malaria Vectors*. InTech Open Access Publisher, Rijeka, Croatia. http://dx.doi.org: 10.5772/56117.

Tananchai, C., R. Tisgratog, W. Juntarajumnong, J. P. Grieco, S. Manguin, A. Prabaripai, and T. Chareonviriyaphap. 2012. Species diversity and biting activity of *Anopheles dirus* and *Anopheles baimaii* (Diptera: Culicidae) in a malaria prone area of western Thailand. Parasites Vectors 5: 211.

Tisgratog, R., C. Tananchai, W. Juntarajumnong, S. Tuntakom, M. J. Bangs, V. Corbel, and T. Chareonviriyaphap. 2012. Host feeding patterns and preference of *Anopheles minimus* (Diptera: Culicidae) in a malaria endemic area of western Thailand: baseline site description. Parasites Vectors 5: 114

Walton. C., J.M. Handley, C. Kuvangkadilok, F.H. Collins, R.E. Harbach, V. Baimai, and R.K. Butlin. 1999. Identification of five species of the *Anopheles dirus* complex from Thailand, using allele-specific polymerase chain reaction. Med. Vet. Entomol. 9: 13: 24–32.

Walton, C., P. Somboon, S.M. O'Loughlin, S. Zhang, R.E. Harbach, Y.M. Linton, B. Chen, K. Nolan, S. Duong, M.Y. Fong, I. Vythilingam, Z.D. Mohammed, H.D. Trung, and R.K. Butlin. 2007. Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. Infect. Genet. Evol. 7: 93–102.

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