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Species abundance and density of malaria vectors in Western Thailand and implications for disease transmission

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ABSTRACT

Understanding the dynamics of malaria vectors and their interactions with environmental factors is crucial for effective malaria control. This study investigated the abundance, species composition, seasonal variations, and malaria infection status of female mosquitoes in malaria transmission and non-transmission areas in Western Thailand. Additionally, the susceptibility of malaria vectors to pyrethroid insecticides was assessed. Entomological field surveys were conducted during the hot, wet, and cold seasons in both malaria transmission areas (TA) and non-transmission areas (NTA). The abundance and species composition of malaria vectors were compared between TA and NTA. The availability of larval habitats and the impact of seasonality on vector abundance were analyzed. Infection with *Plasmodium* spp. in primary malaria vectors was determined using molecular techniques. Furthermore, the susceptibility of malaria vectors to pyrethroids was evaluated using the World Health Organization (WHO) susceptibility test. A total of 9799 female mosquitoes belonging to 54 species and 11 genera were collected using various trapping methods. The number of malaria vectors was significantly higher in TA compared to NTA ($P < 0.001$). *Anopheles minimus* and *An. aconitus* were the predominant species in TA, comprising over 50% and 30% of the total mosquitoes collected, respectively. Seasonality had a significant effect on the availability of larval habitats in both areas ($P < 0.05$) but did not impact the abundance of adult vectors ($P > 0.05$). The primary malaria vectors tested were not infected with *Plasmodium* spp. The WHO susceptibility test revealed high susceptibility of malaria vectors to pyrethroids, with mortality rates of 99–100% at discriminating concentrations. The higher abundance of malaria vectors in the transmission areas underscores the need for targeted control measures in these regions. The susceptibility of malaria vectors to pyrethroids suggests the continued effectiveness of this class of insecticides for vector control interventions. Other factors influencing malaria transmission risk in the study areas are discussed. These findings contribute to our understanding of malaria vectors and can inform evidence-based strategies for malaria control and elimination efforts in Western Thailand.

1. Introduction

Malaria, caused by *Plasmodium* spp. and transmitted through the bites of infected female mosquitoes of the genus *Anopheles*, poses a significant public health burden worldwide. In 2021, there were an estimated 247 million malaria cases globally, resulting in 11.7 million deaths between 2000 and 2021 (WHO, 2022). Thailand has made remarkable progress in malaria control, with a substantial decrease in disease incidence over the past two decades. According to the World Health Organization (WHO) report, malaria incidence in Thailand declined from 3.7 cases per 1000 population in 2002 to 0.2 cases per 1000 population in 2021 (WHO, 2022). Furthermore, the number of malaria cases has been steadily declining each year, with a noteworthy 22.3% reduction between 2020 and 2021 (WHO, 2022).

Malaria transmission in Thailand is predominantly concentrated along the borders with eastern Myanmar, western Cambodia, and northern Malaysia (Chareonviriyaphap et al., 2000). The primary malaria vectors in these regions are *Anopheles dirus*, *An. minimus*, and *An. maculatus* (Zhang et al., 2022). These vectors are responsible for transmitting the two predominant malaria parasite species, *Plasmodium*

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falciparum and *P. vivax* (Zhou et al., 2005). The distribution of malaria cases is primarily influenced by human activities near the forest fringe, where local villagers engage in activities such as hunting, logging, or visiting relatives across the border (Department of Disease Control, 2019a). The increased human presence in these areas, coupled with environmental and climate changes, can have profound effects on vector ecology, behavior, and mosquito population densities.

While malaria transmission persists in many border areas, there are also regions where transmission has been interrupted but the primary malaria vectors remain present. Based on malaria epidemiology, areas can be classified into two categories: transmission areas (TA) and non-transmission areas (NTA). TA include villages with active transmission for at least six months or throughout the year, encompassing both endemic and epidemic areas. NTA are areas where no transmission has been detected for at least three consecutive years, although disease outbreaks may still occur if environmental conditions change. Understanding the variations and potential risks of malaria transmission between transmission and non-transmission areas is crucial for effective malaria control and prevention strategies.

In this study, our objective was to investigate the differences in malaria transmission potential between TA and NTA in Western Thailand. We assessed the abundance and species composition of the malaria vectors in both types of areas, exploring the impact of seasonality. We also assessed the presence of *Plasmodium* spp. infection in the primary malaria vectors. Additionally, we aimed to establish baseline susceptibility levels of field strains of malaria vectors to pyrethroid insecticides in the regions of study. We hypothesized that the variation in malaria transmission potential could be attributed to differences in vector density, which is in turn influenced by factors such as larval habitat availability, environmental conditions, and land use patterns. By examining these factors, we enhanced our understanding of malaria transmission dynamics which will contribute to the development of effective malaria control strategies in Thailand.

2. Materials and methods

To determine the variation and potential risk of malaria transmission in Western Thailand, several aspects were examined, including the seasonal variation of anopheline larval habitats, the density of adult vectors, their infection status, and the susceptibility of female vectors to insecticides.

2.1. Study areas

The study areas were selected from villages in Sai Yok and Sangkhla Buri districts in Kanchanaburi Province and Suan Phueng District in Ratchaburi Province (Table 1, Fig. 1). These areas are situated along the western edge of Kanchanaburi and Ratchaburi provinces, sharing a long border with the neighboring region in Myanmar. The landscape in these areas comprises watersheds, river basins, valleys, tertiary forests, agricultural fields, plantations, and occasional dense clusters of human settlements. The environmental conditions in these areas are suitable for the spread of malaria vectors due to their location in a rain shadow zone. The villages predominantly consist of houses with brick or concrete walls, although some are constructed using wood or bamboo mats. Agriculture serves as the main source of income for the local population, with crops such as rice, palm, sugarcane, and cassava being cultivated. The villages are surrounded by lush vegetation, trees, orchards, cultivated fields, and rice paddies. Water reservoirs are present to provide water during droughts, and there is a network of irrigation systems in some areas for rice cultivation. Livestock and domestic animals, including cattle, buffalo, chickens, goats, pigs, dogs, and cats, are commonly found in the villages and serve as blood sources for zoophilic mosquitoes.

Table 1
The selected transmission and non-transmission areas along Thailand's borders.

Province	District	Area	Village	Latitude	Longitude
Kanchanaburi	Sai Yok	TA	Bong Ti Lang	14.097310	99.000570
Kanchanaburi	Sai Yok	NTA	Sai Thong	14.114490	99.130838
Kanchanaburi	Sangkhla Buri	TA	Morakha	15.212658	98.312845
Kanchanaburi	Sangkhla Buri	NTA	Huay Ma Lai	15.164627	98.340342
Ratchaburi	Suan Phueng	TA	Nong Ta Dang	13.351900	99.249260
Ratchaburi	Suan Phueng	NTA	Bo Kao Bon	13.476255	99.319381

Abbreviations: TA, transmission area; NTA, non-transmission area.

2.2. Adult mosquito collection and Plasmodium spp. detection

Adult anopheline mosquitoes were collected at all six study sites during three different seasons (hot, wet, and cold) from March 2021 to March 2022 using Centers for Disease Control (CDC) miniature light traps baited with dry ice. The hot season is March to June, the wet season is July to the end of October, and the cold season is between November and February. At each site, ten CDC light traps were operated outdoors for three consecutive nights in and around the villages and near the immature survey sites. All traps were placed for approximately 15 h (17:00–8:00 h) and collected the next morning. The collecting cups were immediately placed on dry ice, and the mosquitoes were sexed, counted, identified morphologically using the illustrated keys for mosquitoes of Thailand (Rattanarithikul et al., 2010), and stored at −80 °C for further processing. The categorization of malaria vector status as primary, secondary, or suspected malaria vector in this study adhered to the classification guidelines outlined in the "Guide to Malaria Elimination for Medical and Public Health Personnel in Thailand" (Department of Disease Control, 2019b) as follows: primary vectors (*Anopheles dirus*, *An. minimus* and *An. maculatus*); secondary vectors (*An. epiroticus* and *An. aconitus*); and suspected vectors (*An. barbirostris*, *An. philippinensis*, *An. campestris*, *An. culicifacies*, *An. kochi*, *An. annularis* and *An. sawadwongporni*).

The head and thorax of the vectors from each study site were removed and examined for the presence of *Plasmodium* spp. using a PCR technique (Lee et al., 2015). Briefly, mosquito samples were pooled with approximately 10 samples per pool based on study location and species. Each mosquito sample pool was homogenized using a 3.2 mm steel ball in a mixer mill (Next Advance, USA) to extract the DNA. The extraction solution was made up of 300 µl ATL buffer (Qiagen, Germany), 1 µl carrier RNA, and 20 µl proteinase K (Qiagen, Germany). The homogenates were centrifuged for 3 min at room temperature at 1000 rpm after being incubated overnight at 56 °C in an oscillating thermoblock. A total of 200 µl of each homogenate's supernatant was added to the MagAttract 96 cador Pathogen Kit (Qiagen, Germany), and 100 µl of buffer solution was used to elute the DNA. Real-time PCR was conducted using primers, probes, and reaction conditions as previously described by Lee et al. (2015). All assays were carried out under standard conditions (1 cycle at 95 °C for 5 min; 45 repeated cycles at 95 °C for 30 s and at 60 °C for 30 s) using a CFX96 Real-time PCR machine (Bio-Rad, USA). A cut-off of 40 cycles was applied to define positive samples. The temporal changes in vector density and their infection status between TA and NTA were determined.

2.3. Mosquito larval habitat survey and collection of immature stages

To assess the characteristics of anopheline larval habitats, surveys were conducted to collect the immature stages of mosquitoes in and around the study areas concurrently with adult mosquito collections. The collection and rearing procedures, as well as the description of larval habitats, followed modified guidelines from the Walter Reed

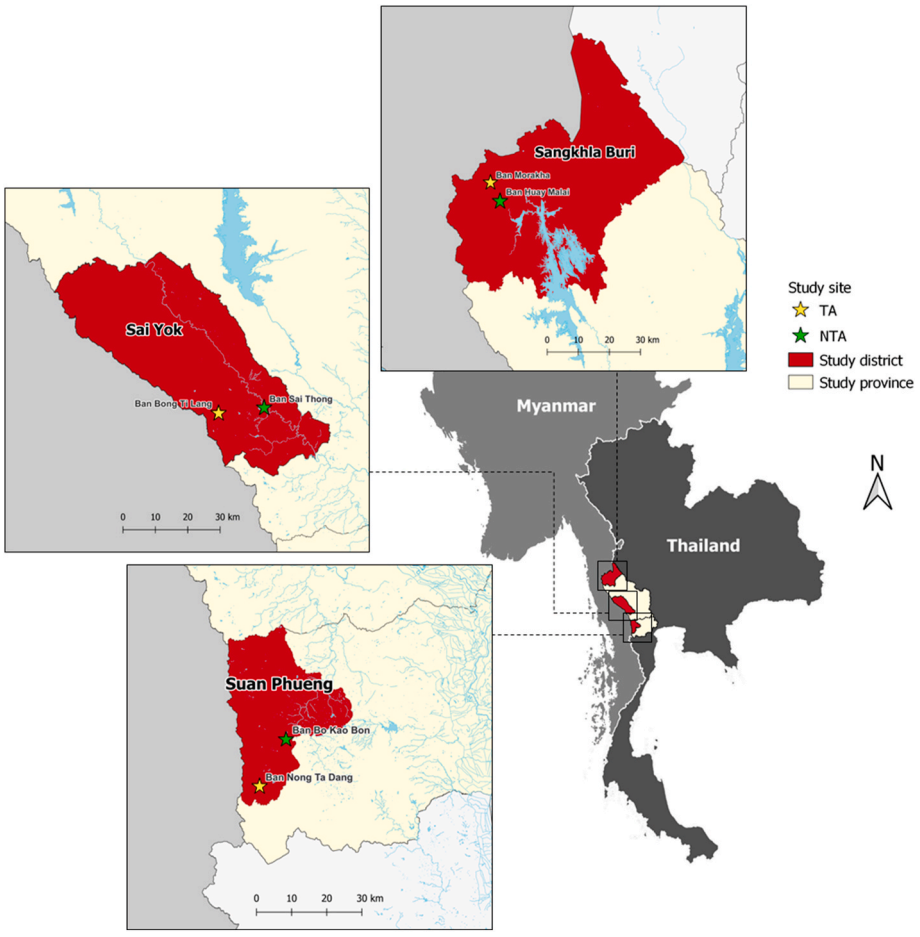

Fig. 1. Maps showing study villages in Sangkhla Buri and Sai Yok Districts in Kanchanaburi Province and Suan Phueng District in Ratchaburi Province.

Biosystematics Unit (WRBU, 1997). The physical and biological characteristics of the larval habitats were recorded, including weather conditions, shade, water movement, water permanency, aquatic vegetation (algae and debris), and habitat type (Fig. 2). Larvae and pupae were sampled from larvae-positive habitats, placed in individual containers, and labeled with the study location. Some of the collected larvae were allowed to develop into adults for further species identification.

2.4. WHO susceptibility bioassay

For the WHO susceptibility bioassay, the discriminating concentration of each pyrethroid insecticide was tested against field strains of malaria vectors to establish baseline susceptibility. Blood-fed female *Anopheles* spp. were collected from the study areas using cow-baited traps and identified. These mosquitoes were then brought to the laboratory and allowed to lay eggs under controlled conditions. The offspring (generation F1) of the wild-caught female mosquitoes, specifically *An. minimus* and *An. aconitus*, was used for the insecticide susceptibility tests.

In the insecticide susceptibility tests, the generation F1 of field strains was used to assess the susceptibility of mosquito populations to pyrethroid compounds. For each exposure time, four replicates of 25 females (3–5 days-old) were exposed to insecticide-impregnated test papers following the WHO guidelines (WHO, 2016). The insecticides tested included deltamethrin (0.05% concentration), permethrin (0.75% concentration), and bifenthrin (0.2% concentration). The mosquitoes were exposed to the insecticide for 1 h, and their knockdown response and mortality were recorded. The knockdown rate was measured after 1 h of exposure, while mortality was assessed 24 h after exposure. The mortality and knockdown rates were calculated to determine the susceptibility status of the mosquitoes to the tested insecticides. The mortality rate was evaluated, and mosquito strains with mortality rates of $\geq$ 98% were considered fully susceptible according to the WHO guidelines (WHO, 2016).

2.5. Data analysis

The abundance of all female mosquitoes, female anophelines, and the malaria vector species collected with CDC light traps was analyzed using a generalized linear mixed model (GLMM) with negative binomial distribution and log link function. Fixed factors were area (TA/NTA), season (hot/wet/cold), and their interactions. Study locations were assigned as a random variable. The effects of area and season factors on the presence of anopheline larvae in larval habitats were analyzed using GLMM with binomial distribution and logit link function. The influence of larval habitat characteristics and habitat type on larval presence was determined using Pearson's chi-square test. Statistical analyses were performed using IBM SPSS version 26. For the WHO susceptibility bioassay, the mortality and knockdown rates were calculated to consider the susceptibility status.

3. Results

A total of 54 mosquito species belonging to 11 genera were collected from 540 traps (Supplementary Table S1). All 9799 female mosquitoes were identified to the species level. The genus *Anopheles* had the highest number of species (19 species), followed by *Culex* (11 species) and *Aedes* (9 species). The remaining genera (*Mansonia*, *Armigeres*, *Uranotaenia*,

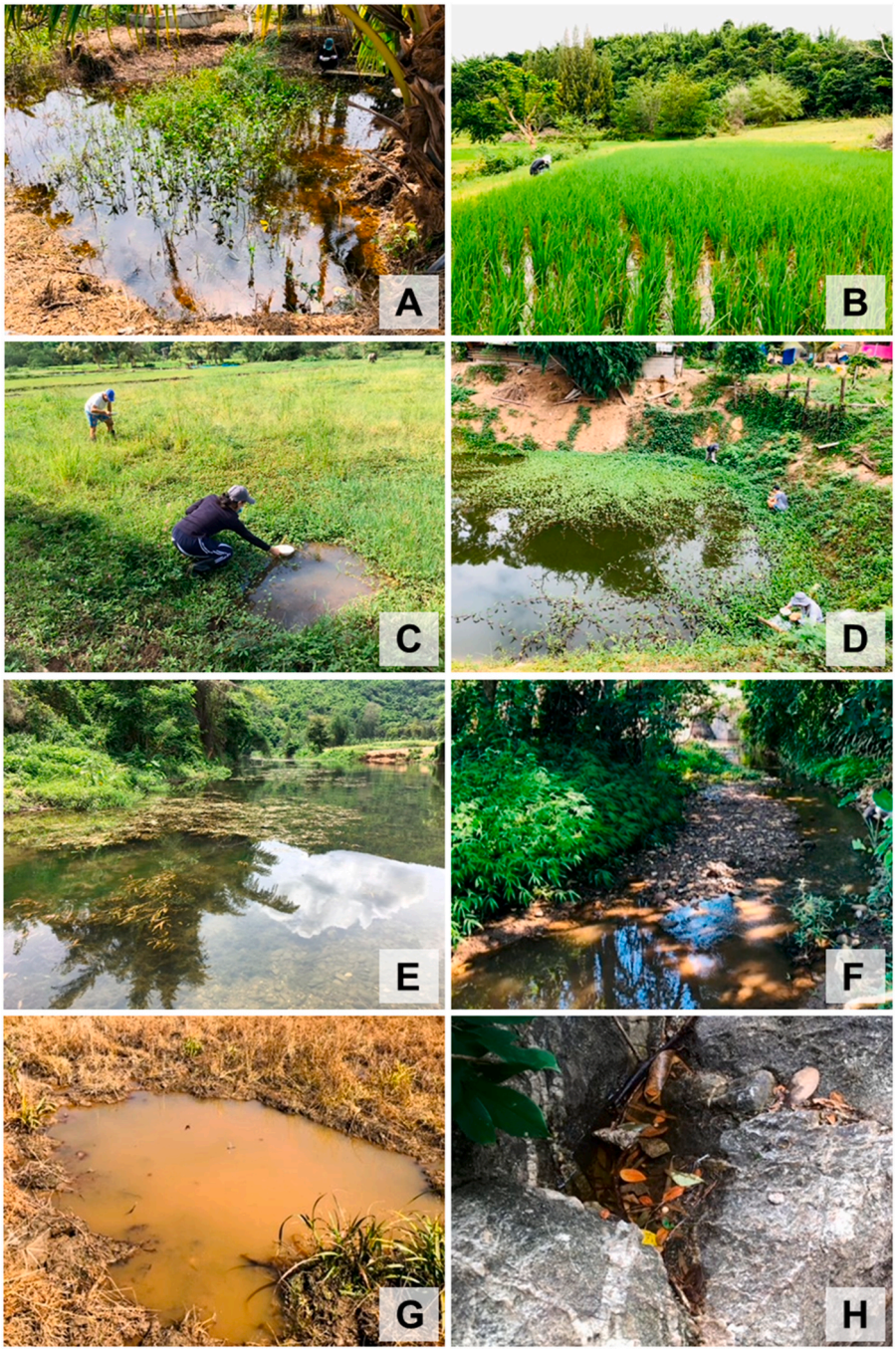

Fig. 2. Temporary (**A**–**C**, **F**–**H**) and permanent (**D**, **E**) mosquito larval habitats. **A**, **C** Marsh. **B** Rice paddy. **F** Stream margin. **G** Pit. **H** Rock pool. **D** Ground pool. **E** Swamp.

Mimomyia, *Coquillettidia*, *Aedeomyia*, *Heizmannia*, *Lutzia*, and *Tripteroides*) each had fewer than five identified species. Among the collected mosquito species, *Mansonia indiana* was the most abundant, accounting for 21.57% of the total mosquitoes collected ($n = 2114$), followed by *Anopheles minimus* (10.66%, $n = 1045$), *Culex vishnui* (8.10%, $n = 794$), *Anopheles aconitus* (6.65%, $n = 652$), and *Aedes albopictus* (6.02%, $n = 590$).

The abundance of all mosquito species, anophelines, and malaria vectors in each season and collecting areas were analyzed (Table 2). Considering the influence of the study area on mosquito abundance, negative binomial GLMM revealed significantly greater abundance of all mosquitoes (rate ratio, RR = 1.76, 95% CI: 1.48–2.09, $P < 0.001$), anophelines (RR = 14.54, 95% CI: 8.72–24.25, $P < 0.001$), and malaria vectors (RR = 18.98, 95% CI: 10.86–33.19, $P < 0.001$) collected in TA compared to NTA. More than 93% of anophelines including malaria vectors were collected from TA, especially in the cold and wet seasons (Table 2).

Significantly more mosquitoes were recorded during the wet season (40.2%) than during the cold season (30.2%) and during the hot season (29.6%) (RR = 1.28, 95% CI: 1.03–1.58, $P = 0.025$, Table 2). However, malaria vector abundance was not affected by changes in seasonality ($P = 0.082$) (Table 2).

Nearly 20%, or 11 species, of the total mosquito population were identified as malaria vectors, including the primary, secondary, and suspected malaria vector species that are considered to be involved in the transmission of malaria (Table 3). All three primary malaria vector

Table 2
Abundance of adult female mosquitoes, anophelines, and malaria vectors collected from all sampling localities during the three seasons.

Season	Area	No. of trap nights	No. of mosquitoes		No. of anophelines		No. of malaria vectors[a]	
			Mean ± SD	%[b]	Mean ± SD	%[b]	Mean ± SD	%[b]
Cold	TA	90	20.86 ± 20.67	63.5	6.79 ± 12.06	93.6	6.39 ± 11.60	94.8
	NTA	90	12.00 ± 15.47	36.5	0.47 ± 0.89	6.4	0.38 ± 0.83	5.2
	Total	180	16.43 ± 18.74	30.2	3.63 ± 9.10	31.3	3.38 ± 8.74	33.0
Hot	TA	90	19.68 ± 32.29	61.0	4.81 ± 6.65	74.8	4.70 ± 6.66	76.9
	NTA	90	12.56 ± 19.47	39.0	1.62 ± 3.01	25.2	1.04 ± 2.12	23.1
	Total	180	16.12 ± 26.83	29.6	3.22 ± 5.39	27.7	2.87 ± 5.26	34.1
Wet	TA	90	32.01 ± 34.81	73.1	8.91 ± 11.51	93.7	8.43 ± 10.75	94.5
	NTA	90	11.78 ± 17.97	26.9	0.60 ± 1.34	6.3	0.46 ± 1.26	5.5
	Total	180	21.89 ± 29.43	40.2	4.76 ± 9.17	41.0	4.44 ± 8.62	33.5
Grand total		540	18.15 ± 25.50		3.87 ± 8.09		3.57 ± 7.72	

Abbreviations: TA, transmission area; NTA, non-transmission area; SD, standard deviation.
[a] Primary, secondary, and suspected malaria vectors.
[b] Percent of total mosquito collection.

Table 3
Abundance of *Anopheles* spp. collected from transmission and non-transmission areas at different study sites.

Species	Sai Yok		Sangkhla Buri		Suan Phueng		Total	% of total *Anopheles* spp.	Malaria vector status
	TA	NTA	TA	NTA	TA	NTA			
An. minimus	284	1	596	111	47	6	1045	50.05	Primary vector
An. maculatus	2		6	1			9	0.43	Primary vector
An. dirus			1	1			2	0.10	Primary vector
An. aconitus	48		11	8	585		652	31.23	Secondary vector
An. annularis				3	1		4	0.19	Suspected vector
An. barbirostris	15	1	16	11	12	1	56	2.68	Suspected vector
An. campestris	6		1	2	11	7	27	1.29	Suspected vector
An. culicifacies				10			10	0.48	Suspected vector
An. kochi	51		4	1	48		104	4.98	Suspected vector
An. philippinensis			1		5		6	0.29	Suspected vector
An. sawadwongporni	3		3	3		2	11	0.53	Suspected vector
An. argyropus					1		1	0.05	
An. jamesii		2	4	3			9	0.43	
An. nivipes					1		1	0.05	
An. peditaeniatus	3	7	3	1	4	3	21	1.01	
An. splendidus			2				2	0.10	
An. tessellatus	1	3			12	1	17	0.81	
An. vagus	1	1			8	44	54	2.59	
An. varuna	11	1					12	0.57	
Anopheles sp.	12	1	10	3	16	3	45	2.16	
Grand total	437	17	658	158	751	67	2088		

Abbreviations: TA, transmission area; NTA, non-transmission area.

species were found in this study (Table 3). *Anopheles minimus* was the predominant species among the anopheline species in almost all sampling areas accounting for 50% (n = 1045) of the total number of *Anopheles* spp. captured, followed by *An. aconitus*, the secondary vector, which accounted for more than 30% (n = 652). These species were widely distributed in the transmission areas, while other suspected vector species were found sparsely throughout the study areas.

A total of 608 primary malaria vectors (67 pools), including *An. dirus*, *An. minimus*, and *An. maculatus*, were examined for the presence of *Plasmodium* spp., but no evidence of pathogen infection was found in any of the mosquito samples examined (Table 4).

The study also examined the larval habitats, as they play a crucial role in determining adult mosquito distribution and abundance. We specifically targeted potential sources of anopheline mosquitoes and

Table 4
Plasmodium spp. detection assays in primary vectors.

Study area	TA			NTA			Total	
Species (strain)	No. tested	No. of pools	PCR result	No. tested	No. of pools	PCR result	No. tested	No. of pools
An. minimus[a]	284	28	Neg	1	1	Neg	285	29
An. minimus[b]	200	20	Neg	78	8	Neg	278	28
An. minimus[c]	33	3	Neg	5	1	Neg	38	4
An. maculatus[a]	2	1	Neg	0	0	Neg	2	1
An. maculatus[b]	3	3	Neg	0	0	Neg	3	3
An. dirus[b]	1	1	Neg	1	1	Neg	1	2
Total	523	56	Neg	85	11	Neg	608	67

Abbreviations: TA, transmission area; NTA, non-transmission area; Neg, negative.
[a] Sai Yok strain.
[b] Sangkhla Buri strain.
[c] Suan Phueng strain.

assessed the percentage of larvae-positive habitats categorized by malaria vectors and primary vectors (Table 5). A total of 136 larval habitats were recorded, with 64.7% ($n = 88$) located in TA and 35.29% ($n = 48$) in NTA. The binomial GLMM revealed significantly more larvae-positive habitats containing malaria vector larvae in TA relative to NTA (RR = 2.90, 95% CI: 1.30–6.47, $P = 0.010$).

Seasonality had an impact on the percentage of larvae-positive habitats. In the context of malaria vectors, fewer larvae-positive habitats were found during the wet season compared to other seasons for malaria vectors (RR = 0.39, 95% CI: 0.16–0.95, $P = 0.038$). Similarly, for primary vectors, the prevalence of larvae-positive habitats was significantly lower in the wet season (RR = 0.14, 95% CI: 0.10–0.18, $P < 0.001$, Table 5).

Table 6 summarizes the larval habitat characteristics of anopheline mosquitoes. The immatures of primary vectors were frequently found in permanent water habitats ($\chi^2 = 4.42$, $df = 1$, $P = 0.036$) that had aquatic vegetation, such as algae and debris ($\chi^2 = 4.57$, $df = 1$, $P = 0.033$). These habitats were often located along the margins of streams ($\chi^2 = 35.32$, $df = 9$, $P < 0.0001$) and were partially shaded from sunlight ($\chi^2 = 11.68$, $df = 2$, $P = 0.003$).

It is worth noting that the species identification of adult mosquitoes that emerged from the sampled larvae corresponded to the same species collected by CDC light traps. This suggests consistency in species composition between the larval and adult stages of the mosquitoes, reinforcing the reliability of the trapping method for studying adult populations (Table 3, Supplementary Table S2).

The results in Table 7 indicate that the mortality rate of all malaria vectors tested in this study was ≥ 99%. This suggests that the mosquito populations tended to be susceptible to pyrethroids. This is an important finding as pyrethroids are commonly used insecticides for malaria vector control. Regarding the knockdown response, bifenthrin showed the weakest knockdown effect among the tested insecticides against *An. minimus* strains from Sangkhla Buri (84%) and Sai Yok (91%), as well as against *An. aconitus* strain from Sangkhla Buri (97%). The knockdown response refers to the ability of the insecticide to quickly immobilize the mosquitoes.

4. Discussion

In this study, our objective was to investigate the differences in malaria transmission potential between transmission and non-transmission areas in Western Thailand. We assessed the abundance and species composition of the malaria vectors in both types of areas, exploring the impact of seasonality. We also assessed the presence of malaria parasite infection in the primary malaria vectors. Additionally, we aimed to establish baseline susceptibility levels of field strains of malaria vectors to pyrethroid insecticides.

We hypothesized that the variation in malaria transmission potential could be attributed to differences in vector density, which is in turn influenced by factors such as larval habitat availability, environmental conditions, and land use patterns. This hypothesis underscores the unique focus of our research, which centers on the interplay between vector density and its contributing factors in shaping malaria transmission dynamics. By examining these multifaceted factors, we aimed to enhance our understanding of malaria transmission, ultimately contributing to the development of more effective malaria control strategies in Thailand. This emphasis on the interrelation of these elements highlights the novelty and significance of our study.

This spatial and temporal study was conducted at six malaria-endemic sites in Western Thailand, where the entomological survey took place in three different seasons within one year. The results provided a catalogue of a variety of mosquito species involved in malaria transmission. Overall, a greater number of mosquitoes, including malaria vectors and other mosquito species, were found in TA than in NTA. The primary vector, *An. minimus*, was found to be the predominant species in TA. According to our observations, the landscape of TA consists of denser, moist forest areas with natural streams, and cultivated crops in mountainous terrain. The surroundings at TA may help create a humid environment with suitable microclimatic conditions for oviposition and reproduction, allowing a malaria vector population to persist throughout the year. The environmental conditions in NTA are more diverse and the forests are fragmented. Apart from this, the improvement of transport networks and deforestation may have changed the environment of small villages towards urbanization, resulting in a decrease of malaria vectors in NTA.

Our findings provide valuable insights into the preferred habitat characteristics of primary malaria vectors. The presence of permanent water bodies with aquatic vegetation, along with shaded areas near marginal streams, appears to be favorable for the development of anopheline larvae. Understanding these habitat preferences can contribute to targeted vector control measures and surveillance efforts aimed at reducing malaria transmission in the study area.

The findings of the present study are in contrast with a previous study conducted in Chiang Mai Province in northern Thailand (Suwonkerd et al., 2002). These authors reported no significant difference in the density of *An. minimus* and *An. dirus* between TA and NTA based on spatial analysis of entomological records collected over a 20-year period from 1977 to 1999. Several factors could contribute to the differences observed between the two studies. First, variations in geographical and environmental conditions between the study sites may influence the biology and ecology of mosquito vectors. The specific characteristics of the landscape, including factors such as vegetation cover, water sources, and land use patterns, can differ significantly between different regions. These variations may create distinct ecological niches that impact mosquito populations differently.

Secondly, differences in trapping techniques used in the two studies could also contribute to variations in mosquito density estimates. In the present study, we used the CDC light traps baited with dry ice for mosquito collection, whereas Suwonkerd et al. (2002) used the indoor and outdoor human landing catch technique. Different trapping methods can yield different results due to variations in trapping efficiency, attractiveness to different mosquito species, and differences in the locations where the traps are deployed. It is important to note that the choice of trapping method can influence the captured mosquito species and may bias the density estimates.

Lastly, the time period of mosquito collection could also play a role in the observed differences. Mosquito populations can exhibit temporal fluctuations in abundance due to various factors such as seasonal variations, climate patterns, and control interventions. The study conducted by Suwonkerd et al. (2002) covered a longer time span, potentially capturing different periods of mosquito population dynamics compared

Table 5
Percentage of larval habitats observed in transmission and non-transmission areas in different seasons.

Season	Area	*n*	Malaria vectors[a]		Primary vectors[b]	
			Percent	95% CI	Percent	95% CI
Cold	TA	33	67	51–84	21.21	7–35
	NTA	13	54	27–81	23	0–46
	Total	46	63	49–77	22	10–34
Hot	TA	25	76	59–93	32	14–50
	NTA	14	50	24–76	43	17–69
	Total	39	67	52–81	36	21–51
Wet	TA	30	50	32–68	13	1–26
	NTA	21	29	9–48	5	-4–14
	Total	51	41	28–55	10	2–18
All seasons	TA	88	64	54–74	22	13–30
	NTA	48	42	28–56	21	9–32
	Total	136	56	48–64	21	14–28

Abbreviations: *n*, number of larval habitats; CI, confidence interval; TA, transmission area; NTA, non-transmission area.

[a] Primary, secondary, and suspected malaria vectors.

[b] Only *An. minimus*, *An. maculatus*, and *An. dirus*.

Table 6
Characteristics of larval habitats with the percentage of malaria vectors and primary vectors presence.

Habitat characteristics	Categories	No. of larval habitats	Malaria vectors (%)[a]	χ^2 test	Primary vectors (%)[b]	χ^2 test
Weather	Clear	102	51.0	$\chi^2 = 5.10$, $df = 2$, $P = 0.078$	22.5	$\chi^2 = 2.64$, $df = 2$, $P = 0.267$
	Cloudy	25	76.0		24.0	
	Shower	9	55.6		0	
Shade	None	66	53.0	$\chi^2 = 1.33$, $df = 2$, $P = 0.513$	9.1	$\chi^2 = 11.68$, $df = 2$, $P = 0.003$
	Partial	59	61.0		33.9	
	Heavy	11	45.5		27.3	
Water movement	Standing water	98	51.0	$\chi^2 = 3.74$, $df = 2$, $P = 0.154$	12.2	$\chi^2 = 18.21$, $df = 2$, $P < 0.0001$
	Slow	25	72.0		40.0	
	Fast	13	61.5		53.8	
Water permanency	Permanent	80	60.0	$\chi^2 = 1.34$, $df = 1$, $P = 0.248$	27.5	$\chi^2 = 4.42$, $df = 1$, $P = 0.036$
	Temporary	56	50.0		12.5	
Aquatic vegetation	Absent	35	40.0	$\chi^2 = 4.82$, $df = 1$, $P = 0.028$	8.6	$\chi^2 = 4.57$, $df = 1$, $P = 0.033$
	Present	101	61.4		25.7	
Habitat type	Ground pool	30	50.0	$\chi^2 = 14.54$, $df = 9$, $P = 0.104$	16.7	$\chi^2 = 35.32$, $df = 9$, $P < 0.0001$
	Flood pool	11	45.5		0	
	Pond/lake	12	33.3		8.3	
	Marsh/swamp	6	66.7		0	
	Stream margin	27	77.8		59.3	
	Stream pool	12	75.0		33.3	
	Rice paddy	5	60.0		20.0	
	Ditch	9	33.3		11.1	
	Wheel track	9	33.3		0	
	Other[c]	15	60.0		6.7	

[a] Primary, secondary, and suspected malaria vectors.
[b] Only *An. minimus*, *An. maculatus*, and *An. dirus*.
[c] Rock pool, pit, animal footprint, cattle wallow, tree hole, stump hole, plant axil, artificial container.

Table 7
Knockdown and mortality of adult females of *An. minimus* and *An. aconitus* field strains induced by pyrethroids.

	Species	Strain	No. tested	KD (*n*)	KD (%)	No. dead	Mortality (%)
Deltamethrin (0.05%)	*An. minimus*	Sai Yok	98	98	100	98	100
	An. minimus	Sangkhla Buri	100	100	100	99	99
	An. aconitus	Sangkhla Buri	99	99	100	99	100
	An. aconitus	Suan Phueng	100	100	100	100	100
Permethrin (0.75%)	*An. minimus*	Sai Yok	97	97	100	97	100
	An. minimus	Sangkhla Buri	100	98	98	100	100
	An. aconitus	Sangkhla Buri	94	94	100	94	100
	An. aconitus	Suan Phueng	96	94	98	96	100
Bifenthrin (0.2%)	*An. minimus*	Sai Yok	95	86	91	95	100
	An. minimus	Sangkhla Buri	100	84	84	100	100
	An. aconitus	Sangkhla Buri	92	89	97	92	100
	An. aconitus	Suan Phueng	95	95	100	95	100
Control	*An. minimus*	Sai Yok	100	0	0	0	0
	An. minimus	Sangkhla Buri	100	0	0	0	0
	An. aconitus	Sangkhla Buri	100	0	0	0	0
	An. aconitus	Suan Phueng	100	0	0	0	0

Notes: KD (*n*), number of knockdown mosquitoes at 1 h of exposure; No. dead, number of dead mosquitoes at 24 h post-exposure.

to the present study. These temporal variations can impact the observed differences in mosquito density between TA and NTA.

In our study, interestingly, the proportion of *An. aconitus*, a primarily zoophilic and exophilic mosquito (Tananchai et al., 2019), of all collected mosquitoes, was higher than that of the primary vectors from the adult collection at TA in Suan Phueng District. In this area, there is a large buffalo shed located in the center of the village, that provides a source of blood for *An. aconitus* throughout the year. A herd of buffalo attracts females of *An. aconitus* to feed and reproduce, as we found the immatures of this species in and around the study site. Although *An. aconitus* prefers to feed on animals, it also readily feeds on humans when target animal hosts are scarce (WRBU, 2021). Given its high density in TA, this species could play a significant role and may contribute substantially to malaria transmission in this study area, which is similar to documented high densities of secondary malaria vectors in the sub-Saharan Africa (*An. coustani*, *An. pharoensis*, *An. aquamosus*, and *An. ziemanni*), which have also shown a potential contribution to malaria transmission (Gillies, 1964; Kamau et al., 2006; Fornadel et al., 2011; Asare et al., 2016; Mustapha et al., 2021).

In malaria control programmes, most secondary vectors with zoophilic, exophilic, and exophagic behaviors are generally not considered important targets for malaria control (Kamau et al., 2006). They may not be exposed to the residual insecticide treatment and are likely to maintain a negligible reservoir of malaria parasites until the population of primary vectors returns after the completion of the malaria control programme (Gillies, 1964). Additionally, local malaria transmission may elevate the contribution of a secondary vector to a primary vector (Mustapha et al., 2021), so routine surveillance is needed to identify the species responsible for malaria transmission. Sufficient data on mixed human-animal feeds, infection rates, and ecology of suspect species should be further investigated in order to develop the most appropriate control techniques.

In our study, seasonality did not affect adult vector density, but significantly affected the number of larvae-positive habitats. These

findings highlight the higher prevalence of positive larval habitats in TA compared to NTA, indicating a potentially higher risk of malaria transmission in the TA. The impact of seasonality on the presence of larvae-positive habitats suggests that certain environmental conditions during the wet season may limit the availability of suitable larval sites for both malaria vectors and primary vectors (*An. dirus*, *An. minimus*, and *An. maculatus*). During the wet season, heavy rains flushed out many immature individuals that resided along the stream margin, resulting in fewer larvae-positive habitats being detected (Ratti et al., 2022). In turn, during the hot season with dry conditions, water accumulations in temporary larval habitats gradually dried up, making temporary larval habitats difficult to find. However, permanent larval habitats, such as large ground pools and marshes, appear to be important larval habitats for maintaining mosquito populations. The highly humid environment at TA allows adult vectors to survive during the hot season. This indicates that malaria transmission could potentially occur year-round at TA and may be one reason for the difference between TA and NTA.

It is also possible that other factors are involved in malaria transmission, such as traditions and lifestyles of villagers, socioeconomic conditions, and mobility of people between borders (Suwonkerd et al., 2004). Transportation from suburban areas to commercial areas to sell agricultural products, visiting relatives across the border, and other commercial purposes may also increase human movement through TA, thereby increasing the risk of malaria transmission (Department of Disease Control, 2019a). In addition, intensive use of pesticides in agriculture by locals and migrants could increase selection pressure for insecticide resistance in mosquito vectors (Overgaard et al., 2015).

In the present study, the adult susceptibility bioassay, which was performed only one time for each strain and insecticide, was limited due to insufficient numbers of mosquitoes. Although resistance to pyrethroids was not detected in malaria vectors, insecticide cross-resistance could develop in vector populations due to repeated use of agrochemicals in orchards and crops cultivated in the vicinity of communities (Overgaard, 2006). To confirm resistance levels in field populations, further larval bioassays should be conducted against pyrethroids and other insecticides, including carbamates and organophosphates commonly used for crop protection. Given all these potential factors, areas and villages where malaria transmission has been reduced or eliminated may experience a resurgence of the disease if determined surveillance and prevention are excluded.

The results of the present study indicate that the mosquito populations in the study area have a high susceptibility to pyrethroid insecticides, which is promising for the effectiveness of insecticide-based control interventions. However, the reduced knockdown response to bifenthrin in certain mosquito strains suggests the need for continued monitoring of insecticide susceptibility to ensure the effectiveness of control strategies and to detect any potential development of resistance in the future.

5. Conclusions

This study provides important insights into the variation and potential risk of malaria transmission in the border areas between Thailand and Myanmar. The findings contribute to the development of valuable early warning systems for assessing malaria transmission risk in these regions. The study highlights the importance of conducting entomological surveys using standardized protocols for mosquito population abundance assessment, especially for long-term studies. The accumulated entomological data generated from such surveys are crucial for analyzing temporal changes in vector abundance and understanding the dynamics of mosquito populations. The results emphasize the significance of considering various factors, including geographical, environmental, climatic, and human factors, in the analysis of malaria transmission. The dataset generated in this study contributes to the understanding of how these factors influence malaria transmission and mosquito populations in high-risk areas. This knowledge is essential for the effective planning and implementation of targeted malaria control strategies. Moving forward, further research and continuous monitoring of mosquito populations and malaria transmission dynamics are necessary to maintain the effectiveness of control interventions. Long-term entomological studies combined with comprehensive data on various influencing factors will aid in the identification of emerging risks and the development of tailored strategies for malaria prevention and control in the border regions of Thailand and Myanmar.

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Ethical approval

The mosquito collection procedures in this study underwent review and approval by the AFRIMS Institutional Animal Care and Use Committee (IACUC), granted under approval number PN20-04.

Declaration of the use of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT, an AI-powered language model, in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed, ensuring its accuracy and relevance. The authors take full responsibility for the content of the publication.

CRediT authorship contribution statement

Thanyalak Fansiri: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Boonsong Jaichapor:** Investigation. **Arissara Pongsiri:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Preeraya Singkhaimuk:** Investigation. **Patcharee Khongtak:** Investigation. **Wachiraphan Chittham:** Investigation. **Nattaphol Pathawong:** Investigation. **Duangkamon Pintong:** Investigation. **Bussayagorn Sujarit:** Investigation. **Alongkot Ponlawat:** Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files.

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Appendix A. Supplementary data

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References

Afrane, Y.A., Bonizzoni, M., Yan, G., 2016. Secondary malaria vectors of sub-Saharan Africa: Threat to malaria elimination on the continent? In: Rodriguez-Morales, A.J. (Ed.), Current Topics in Malaria. IntechOpen, London, UK, pp. 437–490. https://doi.org/10.5772/65359.

Chareonviriyaphap, T., Bangs, M.J., Ratanatham, S., 2000. Status of malaria in Thailand. Southeast Asian J. Trop. Med. Publ. Health 31, 225–237.

Department of Disease Control, 2019a. Guide to malaria elimination for Thailand's local administrative organizations and the health network. Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Thailand. https://malaria.ddc.moph.go.th/downloadfiles/Guide%20to%20Malaria%20Elimination%20for%20Thailand%20LAO_EN.pdf.

Department of Disease Control, 2019b. Guide to malaria elimination for medical and public health personnel in Thailand. Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Thailand. https://malaria.ddc.moph.go.th/downloadfiles/Malaria_Manual/แนวทางการปฏิบัติงานกำจัดโรคไข้มาลาเรีย%20สำหรับบุคลากรทางการแพทย์และสาธารณสุขประเทศไทย.pdf.

Fornadel, C.M., Norris, L.C., Franco, V., Norris, D.E., 2011. Unexpected anthropophily in the potential secondary malaria vectors *Anopheles coustani s.l.* and *Anopheles squamosus* in Macha, Zambia. Vector Borne Zoonotic Dis. 11, 1173–1179. https://doi.org/10.1089/vbz.2010.0082.

Gillies, M.T., 1964. The role of secondary vectors of malaria in north-east Tanganyika. Trans. R. Soc. Trop. Med. Hyg. 58, 154–158. https://doi.org/10.1016/0035-9203(64)90004-5.

Kamau, L., Mulaya, N., Vulule, J.M., 2006. Evaluation of potential role of *Anopheles ziemanni* in malaria transmission in western Kenya. J. Med. Entomol. 43, 774–776. https://doi.org/10.1093/jmedent/43.4.774.

Lee, P.C., Chong, E.T.J., Anderios, F., Lim, Y.A., Chew, C.H., Chua, K.H., 2015. Molecular detection of human *Plasmodium* species in Sabah using PlasmoNex™ multiplex PCR and hydrolysis probes real-time PCR. Malar. J. 14, 28. https://doi.org/10.1186/s12936-015-0542-5.

Mustapha, A.M., Musembi, S., Nyamache, A.K., Machani, M.G., Kosgei, J., Wamuyu, L., et al., 2021. Secondary malaria vectors in western Kenya include novel species with unexpectedly high densities and parasite infection rates. Parasites Vectors 14, 252. https://doi.org/10.1186/s13071-021-04748-9.

Overgaard, H.J., 2006. Malaria mosquito resistance to agricultural insecticides: Risk area mapping in Thailand. Research Report 103. International Water Management Institute, Colombo, Sri Lanka. https://www.iwmi.cgiar.org/Publications/IWMI_Research_Reports/PDF/pub103/RR103.pdf. (Accessed 2 January 2024).

Overgaard, H.J., Suwonkerd, W., Hii, J., 2015. The malaria landscape: Mosquitoes, transmission, landscape, insecticide resistance, and integrated control in Thailand. In: Morand, S., Dujardin, J.P., Lefait-Robin, R., Apiwathnasorn, C. (Eds.), Socio-Ecological Dimensions of Infectious Diseases in Southeast Asia. Springer, Singapore, pp. 123–153. https://doi.org/10.1007/978-981-287-527-3_9.

Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E., Richardson, J.H., 2010. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. Southeast Asian J. Trop. Med. Public Health 41 (Suppl. 1), 1–225.

Ratti, V., Chipman, J.W., Wallace, D.I., 2022. Larval flushing alters malaria endemicity patterns in regions with similar habitat abundance. Curr. Res. Parasitol. Vector Borne Dis. 2, 100080 https://doi.org/10.1016/j.crpvbd.2022.100080.

Suwonkerd, W., Overgaard, H., Tsuda, Y., Prajakwong, S., Takagi, M., 2002. Malaria vector densities in transmission and non-transmission areas during 23 years and land use in Chiang Mai Province, Northern Thailand. Basic Appl. Ecol. 3, 197–207. https://doi.org/10.1078/1439-1791-00108.

Suwonkerd, W., Tsuda, Y., Overgaard, H., Chawprom, S., Tuno, N., Prajakwong, S., Takagi, M., 2004. Changes in malaria vector densities over a twenty-three year period in Mae Hong Son Province, northern Thailand. Southeast Asian J. Trop. Med. Publ. Health 35, 316–324.

Tananchai, C., Manguin, S., Bangs, M.J., Chareonviriyaphap, T., 2019. Malaria vectors and species complexes in Thailand: Implications for vector control. Trends Parasitol. 35, 544–558. https://doi.org/10.1016/j.pt.2019.04.013.

WHO, 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed. World Health Organization, Geneva, Switzerland https://fctc.who.int/publications/i/item/9789241511575.

WHO, 2022. World Malaria Report 2022. World Health Organization, Geneva, Switzerland. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022.

WRBU, 1997. Collecting, rearing, mounting and shipping mosquitoes. Walter Reed Biosystematics Unit, Suitland, MD, USA. https://wrbu.si.edu/docs/mq_crms.pdf. (Accessed 15 February 2023).

WRBU, 2021. *Anopheles aconitus* Donitz, 1902. Walter Reed Biosystematics Unit, Suitland, MD, USA. https://www.wrbu.si.edu/vectorspecies/mosquitoes/aconitus. (Accessed 16 March 2023).

Zhang, C., Yang, R., Wu, L., Luo, C., Yang, Y., Deng, Y., et al., 2022. Survey of malaria vectors on the Cambodia, Thailand and China-Laos Borders. Malar. J. 21, 399. https://doi.org/10.1186/s12936-022-04418-w.

Zhou, G., Sirichaisinthop, J., Sattabongkot, J., Jones, J., Bjørnstad, O.N., Yan, G., Cui, L., 2005. Spatio-temporal distribution of *Plasmodium falciparum* and *P. vivax* malaria in Thailand. Am. J. Trop. Med. Hyg. 72, 256–262.