Trends in Parasitology

Review

Malaria Vectors and Species Complexes in Thailand: Implications for Vector Control

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There are seven *Anopheles* species incriminated as important (primary) malaria vectors in Thailand. These vectors belong to species complexes or are in closely related groups that are difficult to separate morphologically. Precise species identification, using molecular methods, enables control operations to target only important vectors and to increase understanding of their specific ecological requirements, bionomic characteristics, and behavioral traits. This review focuses on adult mosquito behavior, vector transmission capacity, and geographical distribution of malaria vectors in Thailand identified using genetic and molecular identification methods between 1994 and 2019. A better understanding of *Anopheles* biodiversity, biology, behavior, vector capacity, and distribution in Thailand and neighboring countries in the Greater Mekong Subregion (GMS) will facilitate more effective and efficient vector-control strategies and consequently contribute to a further decrease in the malaria burden.

Highlights

Enhanced molecular methods have facilitated the identification of members within the *Anopheles* isomorphic sibling species 'complexes' present in Thailand and other countries comprising the Greater Mekong Subregion (GMS) in Southeast Asia.

Molecular identification methods augment the greater malaria vector-control challenge in the context of outdoor malaria transmission in Thailand and the GMS.

Utilizing accurate vector species identification techniques allows greater focus on remaining gaps in the understanding of key vector mosquito behavior and interrelated human behaviors in malaria endemic countries.

Malaria vector distribution, bionomics, and vector capacity are critical for identifying and implementing targeted and more effective vector-control strategies in Thailand and the GMS.

Status of Malaria and Its Vectors

Among mosquito-borne diseases, malaria remains the most prevalent and a major public health priority in many tropical and subtropical regions. An estimated 3.7 billion people in 90 endemic countries and territories are at risk [1]. In 2018, over 200 million cases of malaria were reported worldwide, resulting in an estimated 429 000 deaths. Over 90% of morbidity and mortality occurred in sub-Saharan Africa, with deaths predominantly among children under 5 years of age and pregnant women [1]. The Southeast Asian region accounted for around 5% of the estimated global morbidity/mortality burden [1]. Since 2010, the Southeast Asian countries, collectively, have seen significant reductions in the overall malaria burden, with 59% and 54% decreases in reported malaria cases and mortality, respectively [1,2]. Today, **malaria elimination** (see Glossary) is a primary strategic goal for many countries, including Thailand [3], the aim being to achieve a complete interruption of all autochthonous transmission in defined geographic areas. Yet the incidence of malaria remains stubbornly high in some locations and countries despite substantial efforts and resources used to combat it [1]. Specific strategies for achieving elimination vary by country and locality, and are influenced by multiple epidemiological, financial, and political factors [4].

Typically, four species of *Plasmodium* infect humans in the Southeast Asian region, with *Plasmodium falciparum* and *Plasmodium vivax* being predominant, followed by sporadic cases of *Plasmodium malariae* and *Plasmodium ovale*. More recently, *Plasmodium knowlesi*, a zoonotic Asian simian malaria parasite, has been found to infect humans with increasing frequency [5]. Malaria parasites are naturally transmitted to humans by the bite of the infective female *Anopheles* mosquitoes. Of the currently known *Anopheles* species, of which at least 540 are described or are as yet to be formally named, approximately 80 are considered important public health vectors of malaria and lymphatic filariasis [6]. Of these, 41 species are deemed 'dominant' or primary malaria

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https://doi.org/10.1016/j.pt.2019.04.013

vectors, of which many are grouped within assemblages called **species complexes** [7]. Globally, there are at least 25 known *Anopheles* species complexes, which include approximately 135 sibling species, the majority of which are present in Asia [8–10]. These complexes are comprised of closely related and phenotypically indistinguishable (isomorphic) 'sibling' species. Within a complex, members can present different epidemiological roles in malaria transmission, ranging from important primary vectors to being of only secondary (incidental) concern, or nonvectors. Additionally, some sibling members may have overlapping spatial distributions (sympatry), with each species, presumably, separated by differing behaviors and bionomics that might preclude or diminish opportunities for cross-mating (although natural hybridization can occur). Thus, accurate identification, allowing different sibling species to be distinguished, is important in order to understand and better define local transmission dynamics, and for applying **vector control** [11].

The ecology and behavior of malaria vectors is a key determinant for selecting the most appropriate and efficacious intervention methods to reduce transmission risk. Currently, two primary control interventions to combat the *Anopheles* vector include the application of insecticides on the inside surfaces of houses (i.e., **indoor residual spraying** or **IRS**) and the use of **long-lasting insecticide-treated nets (LLINs)** [1,12]. Unfortunately, neither method has an appreciable impact on reducing outdoor transmission [13]. Thus, we need first to quantify the contribution of outdoor transmission, and then characterize the degree of spatial heterogeneity in order to develop site-targeted tools to counter the risk of transmission. The development of effective vector-control strategies that address all aspects of transmission risk is challenging but is necessary in order to achieve a long-term, sustainable reduction in malaria.

Malaria in Southeast Asia and Thailand

Approximately 460 million people reside in malaria-endemic areas in Southeast Asia, representing 9 of the 11 countries in the region [1]. In 2017, approximately 1.24 million confirmed malaria cases were reported, while malaria-related deaths decreased by 88% between 2010 (2421) and 2016 (299). India and Indonesia, the two largest countries by population, collectively accounted for 98.6% of all reported infections (85% and 13.6%, respectively), and approximately 90% of malaria deaths [1]. Six countries (Bangladesh, Bhutan, Democratic People's Republic of Korea, Nepal, Timor-Leste, and Sri Lanka) reported a greater than 75% decrease in malaria incidence between 2000 and 2014. The Maldives and Sri Lanka were designated 'malaria-free' areas in 2015 and 2016, respectively [1]. In 2018, only 22 malaria-related deaths were reported from six countries (Cambodia, China, Lao PDR, Myanmar, Thailand, and Vietnam) in the **Greater Mekong Subregion (GMS)** [14]. While several countries have made substantial progress in reducing or eliminating malaria transmission (e.g., Bangladesh, Bhutan, China, Nepal, Timor-Leste, and Sri Lanka), others still struggle to contain the disease more broadly or in specific refractory areas [1]. Countries working towards elimination and a 'malaria-free' status are China by 2020, Nepal, Timor-Leste, and Thailand by 2025, and Bangladesh, Myanmar, Indonesia, and India by 2030 [1]. Some countries, however, face greater challenges than others might. For example, in 2017 Cambodia reported a significant setback of nearly 50 000 malaria cases, a 95% increase from 2016. Contributing factors include changing environmental conditions, human population movement, and increasing expansion of drug-resistant malaria parasites [15]. Additionally, vector resistance to common insecticides presents another major challenge, potentially having a significant impact on the effectiveness of IRS and LLINs. Fortunately, the majority of important malaria vectors in Southeast Asia currently show no or low levels of resistance compared with the vectors in Africa [16–18].

In Thailand, malaria remains focally entrenched, especially along rural stretches of border areas with Myanmar, Cambodia, and Malaysia [19]. In recent decades, surveillance data from the Thai Ministry of Public Health show nationally that malaria peaked in 1981, with 473 210 confirmed cases. Cases steadily declined thereafter to 37 209 (0.5 cases/100 000 population) in

Glossary

Allele-specific PCR (AS-PCR): a molecular technique used to identify sibling (isomorphic, 'cryptic') species within certain *Anopheles* species complexes based on specific primers for each member.

Anthropophily: mosquitoes showing a greater preference for feeding on humans compared with available animal hosts.

Circumsporozoite protein enzyme-linked immunosorbent assay (CSP-ELISA): a test to detect sporozoite infections in *Anopheles* mosquitoes.

Endophagy: greater tendency of mosquitoes to blood feed on hosts inside structures (e.g., human habitations).

Exophagy: greater tendency of mosquitoes to blood feed on hosts outside structures.

Exophily: greater propensity of mosquitoes to rest outdoors, usually quantified as the proportion of mosquitoes resting outdoors versus indoors.

Great Mekong Subregion (GMS): an area that includes six countries: Cambodia, (southern) China, Lao PDR, Myanmar, Thailand, and Vietnam.

Host preference: greater propensity of female mosquitoes to blood feed on a specific host species or group (e.g., mammalian versus avian).

Indoor residual spraying (IRS): a vector-control intervention based on the application of insecticides on the inside surfaces of houses.

Internal transcribed spacer 2 (ITS2): a ribosomal DNA (rDNA) widely used to differentiate cryptic (isomorphic) species of *Anopheles*.

Long-lasting insecticide-treated nets (LLINs): nets used as a personal protection device (more broadly, vector control) against mosquito bites for those sleeping under these nets.

Malaria elimination: interruption of local transmission (reduction to zero incidence) of a specified malaria parasite in a defined geographical area as result of deliberate control activities. Continued measures to prevent re-establishment of transmission are required.

Residual transmission: the persistence of malaria transmission over distribution of LLINs and/or application of IRS when vectors are susceptible to the insecticides. A combination of vector bionomics and human behaviors may compromise control measures, 2014 (Figure 1). Notwithstanding a modest increase in 2013 (52 808 cases), 2017 saw slightly fewer than 14 000 cases, and malaria-related mortality declined to 0.02/100 000, both representing all-time lows for Thailand [20]. This significant reduction in cases and mortality is credited to ongoing diagnostic activity (active and passive mechanisms) and a treatment network combined with well-organized vector control deploying site-specific periodic IRS and LLIN distribution [20].

In Thailand today, *P. vivax* is the most commonly microscopically diagnosed causal agent of malaria, when excluding mixed-parasite infections; it represents 83.5% of all cases compared to *P. falciparum* (~5:1 ratio) [20]. *Plasmodium knowlesi* was first reported in humans in Thailand in 2004 in Prachuap Khiri Khan Province near the Myanmar border [21]. A total of 33 *P. knowlesi* infections were identified between 2006 and 2009 in Tak, Chantaburi, Prachuap Khiri Khan, Yala, and Narathiwat provinces [22]. Understandably, this parasite is commonly misdiagnosed, thus underestimating its true contribution to human infections [5,23].

Despite the significant reduction in malaria cases in Thailand, serious challenges remain for achieving full malaria elimination in endemic remote rural areas, due, in part, to frequent and uncontrolled human population movements and trade near the international borders with Myanmar, Cambodia, Laos, and Malaysia. The recent upsurge in cases along the Thai–Malaysia border and the southern provinces of Pattani, Yala, Narathiwat, and Songkhla (approximately 15 million people at risk) was reported between 2015 and 2017; these provinces represented 18.9% (in 2015) and 33.7% (in 2017) of all cases nationwide [20].

Malaria Vectors in Thailand and Other GMS Countries

Theobald [24] published one of the earliest monographs on the Culicidae, including reference to *Anopheles* mosquitoes in Thailand. In 1923, Barnes was the first to document the role of *Anopheles* in the transmission of malaria in Thailand [25]. In 1959, the first checklist of the Culicidae in Thailand was published; it included 47 *Anopheles* species [26]. Nearly a decade later, 52 species of *Anopheles* were listed as being present in Thailand [27], followed by 72 species in 1990 [28], including four unnamed anophelines. In 2006, 73 species of *Anopheles* were recorded, of which 71 were formally named taxa [29]; they included *Anopheles harrisoni* (formerly *Anopheles minimus* C). In 2011, Somboon *et al*. [30] described *Anopheles rampae*, formerly *Anopheles maculatus* chromosomal form K, and in 2013, 73 formally named species were documented in Thailand [31]. More recently, five new species, including *Anopheles prachongae* (Gigas Complex), and four new members in the Barbirostris Complex (*Anopheles dissidens*, *Anopheles saeungae*, *Anopheles wejchoochotei,* and *Anopheles barbirostris* sp. 3) were described following the earlier molecular and cytogenetic evidence [32,33]. There are currently 77 formally named *Anopheles* species and two unnamed putative species that occur in Thailand.

Of the 79 *Anopheles* taxa, generally only seven are regarded as important malaria vectors in Thailand [10], while some other species circumstantially may play more minor or secondary roles as vectors. The primary vector species include (i) *Anopheles baimaii* Sallum & Peyton (previously *Anopheles dirus* D) [34], (ii) *An. dirus* Peyton & Harrison [35], (iii) *An. minimus* Theobald (previously species A) [29], (iv) *An. maculatus* Theobald [36], (v) *Anopheles sawadwongporni* Rattanarithikul & Green [37], (vi) *Anopheles pseudowillmori* (Theobald) [38], and (vii) *Anopheles aconitus* Doenitz [39].

Sibling species, by definition, are very difficult, if not impossible, to separate using morphological characters alone [40,41]. The ability to differentiate isomorphic species is important as some species serve as efficient malaria vectors with high **vectorial capacity**, while others are not deemed epidemiologically important. Often the differences between a 'good' vector species and a

including early and/or outdoor biting of mosquitoes and human activity away from protected areas.

Restriction fragment length polymorphism PCR (RFLP-PCR): restriction fragment length polymorphism-polymerase chain reaction, a molecular technique in which DNA samples are digested into fragments by one or more restriction enzymes and amplified for detection.

Sequence-characterized amplified region PCR (SCAR-PCR): a molecular technique derived from the molecular cloning of the amplified DNA fragments from random amplified polymorphic DNA (RAPD).

Species complex: an assemblage ('complex') of closely related and phenotypically indistinguishable (isomorphic) species ('siblings') that may present different epidemiological roles in pathogen transmission ranging from being important vectors to nonvectors.

Vector control: deliberate measures of any kind directed against malaria-transmitting mosquitoes intended to limit their ability to transmit pathogens.

Vectorial capacity: the number of new infections that a population of a given vector species would induce, per case per day, at a given place and time, assuming that the human population is, and remains, fully susceptible to malaria.

Zoophily: mosquitoes having a greater attraction/preference for feeding on animal hosts compared with humans.

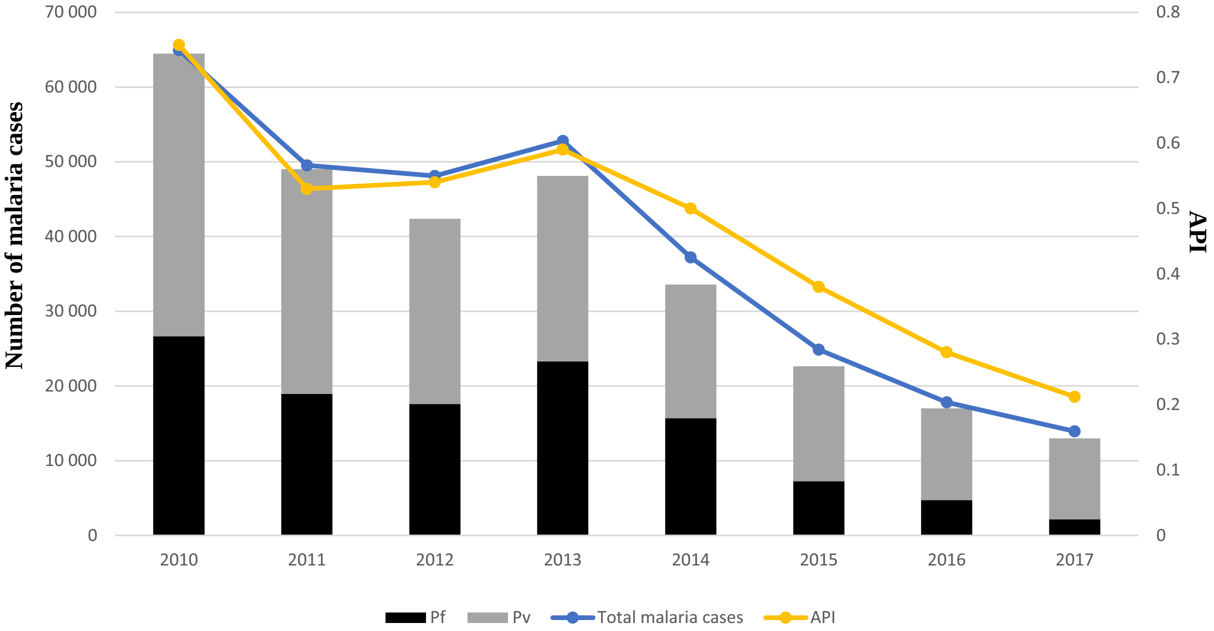

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Figure 1. Number of reported malaria infections and annual parasite incidence in Thailand from 2010 to 2017. The figure shows an estimated 75% decline in malaria-related morbidity (>14 000 cases) and mortality (0.02/100 000). Data obtained from the annual reports 2010–2017, Bureau of Vector-Borne Diseases, Ministry of Public Health, Thailand. Abbreviations: API, annual parasite incidence, cases per 1000 population; Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*.

relatively 'poor' one is predicated on parasite receptivity (competence), feeding behavior, **host preferences**, and longevity (survival) that potentially result in a greater likelihood of contact with humans and a longer expectation of infective life, thereby increasing the probability or capacity to transmit parasites [42–44]. *Anopheles* are sometimes referred to as either primary or secondary (incidental) malaria vectors [7,45,46]. The incrimination of primary vectors is usually clear-cut; they commonly feed on humans, are often abundant, and have measurable sporozoite rates in the population. Incrimination of secondary vectors is more complicated because these species may be relatively uncommon most of the time, are infrequent feeders on humans (opportunistic behavior), and are seldom found harboring sporozoites. However, they may be seasonally abundant and play a more significant temporal role in transmission for short periods. Adult mosquito behavior and larval ecology may also differ significantly between primary and secondary vectors, thus requiring different measures to monitor and control them.

Although some observations exist in Thailand on species-specific trophic behavior and biting patterns within complexes, most published studies are based solely on phenotypic species identification [thus, reported as '*sensu lato*' (s.l.)]. This has resulted in uncertainty on the actual species under observation [47,48]. More recently, molecular techniques that reliably distinguish between sibling species and close members within groups have been used in investigations. These include techniques such as **allele-specific PCR (AS-PCR)**, **sequence-characterized amplified region (SCAR-PCR)**, **restriction fragment length polymorphism (RFLP-PCR)**, and others [45,49–53]. This review includes most published studies in Thailand and the GMS relevant to *Anopheles* vector species identified using molecular identification techniques.

Dirus Complex

Dirus Complex currently contains eight species. Five species, *An. baimaii*, *An. dirus*, *Anopheles cracens*, *Anopheles scanloni*, and *Anopheles nemophilous*, are present in Thailand; the first two are regarded as primary malaria vectors in that country [10,29]. *Anopheles dirus* is widespread throughout Thailand, whereas *An. baimaii* is more limited in distribution [10,29]. Both species can occur in sympatry and predominately inhabit forest and forest-fringe regions. The Dirus Complex members (i.e., *An. dirus* s.l.) show a greater tendency toward **exophily** [46,54,55].

Table 1. Behavioral Traits of Primary and Secondary Malaria Vectors in Thailand Identified by Molecular Methods[b]

Species (Complex or Group)	Location	Tropism	Trophic behavior	Peak biting times (hours)	Species identification method	Refs
Anopheles dirus[a] (*Dirus Complex*)	Wang Thong, Phitsanulok	Anthropophilic	Exophagic	21:00–22:00	DNA probes	[34]
	Makaam, Chantaburi	Anthropophilic	Exophagic	21:00–24:00	Species-specific DNA probes	[58]
	Sai Yok, Kanchanaburi	Zoophilic	Exophagic	19:00–20:00	AS-PCR	[55]
	Ko Chang, Trat	Anthropophilic	Exophagic	ND	AS-PCR	[77]
	Mae Sot, Tak	Anthropophilic	Endophagic	19:00–20:00	AS-PCR	[56]
	Ubon Ratchathani	Anthropophilic	Exophagic	21:00–23:00	AS-PCR	[120]
	Umphang, Tak	Anthropophilic	Exophagic	23:00–24:00	AS-PCR	[57]
Anopheles baimaii[a] (*Dirus Complex*)	Plai Phraya, Krabi	Anthropophilic	Exophagic	02:00–03:00	DNA probes	[34]
	Sai Yok, Kanchanaburi	Anthropophilic/ Zoophilic	Exophagic	24:00–01:00	AS-PCR	[55]
	Sop Moei, Maehongson	Anthropophilic	Exophagic	01:00–02:00	AS-PCR	[56]
	Mae Sot, Tak	Anthropophilic	Exophagic	21:00–22:00	AS-PCR	[56]
Anopheles cracens (*Dirus Complex*)	Tamot, Phatthalung	Anthropophilic	Exophagic	19:00–21:00	DNA probes	[34]
Anopheles scanloni (*Dirus Complex*)	Thung Yai, Nakhon Si Thammarat	Anthropophilic	Exophagic	19:00–20:00	DNA probes	[34]
Anopheles minimus[a] (*Minimus Complex*)	Sai Yok, Kanchanaburi	Zoophilic	Exophagic	ND	AS-PCR	[121]
	Mae Sot, Tak	Anthropophilic	Exophagic	23:00–24:00	AS-PCR	[122]
	Ko Chang, Trat	Anthropophilic	Endophagic	ND	AS-PCR	[77]
	Sop Moei, Maehongson	Zoophilic	Exophagic	20:00–21:00	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	21:00–22:00	AS-PCR	[56]
	Umphang, Tak	Zoophilic	Exophagic	23:00–24:00	AS-PCR	[57]
Anopheles harrisoni (*Minimus Complex*)	Sai Yok, Kanchanaburi	Zoophilic	Exophagic	19:00–21:00	AS-PCR	[121]
Anopheles aconitus[a]	Sop Moei, Maehongson	Zoophilic	Exophagic	ND	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	ND	AS-PCR	[56]
	Umphang, Tak	Zoophilic	Exophagic	ND	AS-PCR	[57]
Anopheles varuna	Sop Moei, Maehongson	Zoophilic	Exophagic	ND	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	ND	AS-PCR	[56]
Anopheles maculatus[a] (*Maculatus Group*)	Ko Chang, Trat	Zoophilic	Exophagic	ND	AS-PCR	[77]
	Sop Moei, Maehongson	Zoophilic	Exophagic	19:00–20:00	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	22:00–23:00	AS-PCR	[56]
	Umphang, Tak	Zoophilic	Exophagic	19:00–20:00	AS-PCR	[57]
Anopheles sawadwongporni[a] (*Maculatus Group*)	Ko Chang, Trat	Zoophilic	Exophagic	ND	AS-PCR	[77]
	Sop Moei, Maehongson	Zoophilic	Exophagic	22:00–23:00	AS-PCR	[56]
	Mae Sot, Tak	Anthropophilic	Exophagic	19:00–20:00	AS-PCR	[56]
	Umphang, Tak	Zoophilic	Exophagic	19:00–20:00	AS-PCR	[57]
Anopheles pseudowillmori[a] (*Maculatus Group*)	Sop Moei, Maehongson	Anthropophilic	Exophagic	19:00–20:00	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	ND	AS-PCR	[56]
Anopheles dravidicus (*Maculatus Group*)	Sop Moei, Maehongson	Zoophilic	Exophagic	ND	AS-PCR	[56]
	Mae Sot, Tak	Zoophilic	Exophagic	ND	AS-PCR	[56]
Anopheles epiroticus (*Sundaicus Complex*)	Mueang Rayong, Rayong	Anthropophilic	Exophagic	21:00–24:00	AS-PCR	[98]
	Ko Chang, Trat	Zoophilic	Exophagic	18:00–19:00	AS-PCR	[77]

[a] Primary malaria vectors in Thailand.

[b] ND, not determined/reported.

Table 2. *Plasmodium* Infections in *Anopheles* Species in Thailand[a]

Vector species (Complex or Group)	Malaria vector	Sporozoite infections (%)	*Plasmodium*	Location site (District, Province)	Species identification methods (vector/parasite)	Refs
Anopheles dirus (Dirus Complex)	Primary	1.3 (1/72) 0.8 (1/114) 1.6 (29/1744) 1.3 (10/768) 6.4 (7/110) 4.8 (1/21)	PF PF PF, PV PF, PV PF, PV PF	Sisaket Mae Sariang, Maehongson Makaam, Chantaburi Makaam, Chantaburi (Mae-tao-kee) Mae Sot, Tak Hua-Hin, Prachuap Khiri Khan	Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/immunofluorescent antibody assay (IFA) and ELISA DNA probes/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA	[59] [61] [35] [58] [38] [60]
Anopheles baimaii (Dirus Complex)	Primary	2.0 (1/53)	PF	(Mae-tao-kee) Mae Sot, Tak	Phenotypic/CSP-ELISA	[38]
Anopheles minimus (Minimus Complex)	Primary	1.5 (5/342) 0.5 (1/212) 0.5 (5/893) 1.5 (2/137) 0.1 (2/1685) 0.14 (5/3410) 0.19 (9/4632) 5 (1/20) 0.09 (1/1090) 0.66 (8/1204)	PF, PV PF PF, PV PF PF, PV PF PF, PV PV PV PV	Tha Song Yang, Tak Makaam, Chantaburi Hua-Hin, Prachuap Khiri Khan Nong Ya Plong, Phetchaburi Phato, Chumphon Mae Sariang, Maehongson Sangkhlaburi, Kanchanaburi Pak Tho, Ratchaburi Mae Sot, Tak Tha Song Yang, Tak	Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA PCR/single-round multiplex-PCR PCR/real-time PCR Phenotypic/CSP-ELISA	[123] [58] [60] [60] [60] [61] [89] [80] [56] [124]
Anopheles aconitus	Primary	4.7 (1/21)	PF	Phato, Chumphon	Phenotypic/CSP-ELISA	[60]
Anopheles maculatus (Maculatus Group)	Primary	0.2-1.6 1.5 (2/131) 3.1 (1/32) 0.2 (1/445) 0.25 (3/1201) 0.31 (2/640)	ND PF PF PF PF PF, PV	Pakchong, Nakhon Ratchasima Hua-Hin, Prachuap Khiri Khan Tha Sae, Prachuap Khiri Khan Sariang, Maehongson Sangkhlaburi, Kanchanaburi Thasongyang, Tak	Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA	[125] [60] [60] [61] [89] [124]
Anopheles sawadwongporni (Maculatus Group)	Primary	1.1 (5/439) 0.1 (2/1506) 0.16 (2/1248)	PF PF, PV PF, PV	Hua-Hin, Prachuap Khiri Khan Mae Sariang, Maehongson Sangkhlaburi, Kanchanaburi	Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA	[60] [61] [89]
Anopheles pseudowillmori (Maculatus Group)	Primary	0.5 (2/384)	PF, PV	(Mae-tao-kee) Mae Sot, Tak	Phenotypic/CSP-ELISA	[38]
Anopheles campestris *Anopheles barbirostris* (Barbirostris Complex)	Potential	0.42 (2/478) 1.9 (2/105)	PV PV	Sangkhlaburi, Kanchanaburi Thasongyang, Tak	Phenotypic/CSP-ELISA Phenotypic/CSP-ELISA	[89] [124]
Anopheles epiroticus (Sundaicus Complex)	Secondary	0.97 (9/926)	PF, PV	Mueang Rayong, Rayong	PCR/Nested PCR and real-time PCR	[98]

[a] ND, not determined/reported; PF, *Plasmodium falciparum*; PV, *Plasmodium vivax*.

Using molecular identification (e.g., DNA probes and AS-PCR), *An. dirus* and *An. baimaii* show high **anthropophily** with a greater predilection for outdoor biting (**exophagy**). However, one population of *An. dirus sensu stricto* (s.s.) from Kanchanaburi Province has displayed greater **zoophily**, while another from Tak Province, also in western Thailand, has shown stronger **endophagy** (Table 1) [55,56], indicating geographical heterogeneity in behavior across its distribution. *Anopheles dirus* has shown a preference to feed immediately after dusk until midnight [55–57], while *An. baimaii* demonstrated peak biting periods just after midnight until dawn, except for a population from Mae Sot, Tak Province [56] (Table 1). The detection of natural sporozoite infections in *An. dirus* and *An. baimaii*, based on the **circumsporozoite protein enzyme-linked immunosorbent assay (CSP-ELISA)**, has been made on molecularly identified populations [56,58]. Overall, published infection rates (Table 2) for the Dirus Complex have ranged between 0.8% and 6.4% [35,38,58–61].

Plasmodium knowlesi sporozoites were detected in *An. cracens* collected by human-landing and monkey-baited collections in southern Thailand; it is a species that also occurs in peninsular Malaysia and northern Sumatra, Indonesia [62,63]. *Anopheles dirus* is also a vector of *P. knowlesi* in Thailand and southern Vietnam [64]. Currently, five species of *Anopheles* mosquitoes have been incriminated as vectors of *P. knowlesi* in Malaysia, including *Anopheles balabacensis*, *An. cracens, Anopheles introlatus, Anopheles latens*, and *Anopheles hackeri*; all belong to the Leucosphyrus Group [65]. *Anopheles scanloni* and *An. nemophilous* are confined to areas of western and southern Thailand, generally with patchy distributions [10,66]. *Anopheles cracens* and *An. scanloni* are regarded as anthropophilic and appear typically to feed outdoors during the early evening hours (Table 1) [34]. The distribution of *An. nemophilous* occurs along the southern border with Myanmar, western, southeastern and southern Thailand, and peninsular Malaysia [46]. Little is known about the biology of this species, and it is not regarded as a malaria vector of importance.

In Lao PDR, Vietnam, Cambodia, and Myanmar, *An. dirus* and *An. baimaii* have shown opportunistic feeding behavior on humans either indoors or outside dwellings. Peak biting activity is generally between early evening and midnight [44,67–71]. Natural sporozoite infections have ranged between 0.17 and 2.7% of sampled mosquitoes [43,44]. In Myanmar, relatively high sporozoite infections (6.25%) for *An. baimaii* were reported [72], while in northeast India *An. baimaii* was sporozoite-infected in 1.9% of the population sampled [73].

Funestus Group

This large and geographically diverse group contains five subgroups, three of which occur in Thailand, namely, the Aconitus, Culicifacies, and Minimus Subgroups [50]. The Minimus Subgroup includes the Minimus Complex that comprises five sibling species previously identified as A, B, C, D, and E [46,50], three of which have been formerly named, *An. minimus* (= species A), *An. harrisoni* (= species C) Harbach & Manguin, and *Anopheles yaeyamaensis* (= species E) Somboon & Harbach. The first two species are present in Thailand [74], whereas *An. yaeyamaensis* is only known from Ishigaki Island, Japan [75].

An. minimus is a widely distributed and important malaria vector in Thailand [10,76]. *An. harrisoni* appears restricted to small areas in western Thailand, specifically in two districts of Kanchanaburi Province and one district in Chiang Mai Province [29]. This species is sympatric with *An. minimus* in Kanchanaburi Province [10]. Both species are primarily zoophilic when presented host options and demonstrate stronger exophagic behavior. The one exception is from Ko Chang Island, Trat Province, where *An. minimus* exhibited stronger anthropophily and a preference for endophagy [77].

Three closely related species within the Aconitus Subgroup are present in Thailand: *An. aconitus*, *Anopheles varuna*, and *Anopheles pampanai* [10]. *An. aconitus* and *An. varuna* are more common along the Thai–Myanmar border and display greater zoophily with a tendency toward exophily. *An. pampanai* appears restricted to the Thai–Cambodian border area and very little is known about its biology and behavior in Thailand. In Vietnam, *An. pampanai* was reported as a secondary malaria vector, with a *P. vivax* infection rate ranging between 1.5% and 20%, the higher percentage being due to the low sample size (one infected out of five collected) [78,79]. Nearly all studies on natural sporozoite infections within the Minimus Complex (*An. minimus*) and *An. aconitus* were obtained from mosquitoes identified using morphological characteristics alone. The two exceptions that used molecular identification for *An. minimus* are Eamkum *et al*. [80] and Tainchum *et al*. [56], reporting sporozoite infection rates of 0.09% and 5%, respectively (Table 2).

Several studies from Lao PDR (*An. minimus*), Vietnam (*An. harrisoni*), Cambodia (*An. minimus* and *An. aconitus*), and Myanmar (*An. minimus*) used molecularly identified samples [70,72,81]. *An. minimus* exhibited either anthropophilic or zoophilic behavior, with varying biting periods during the evening, and sporozoite infections ranging between 1.4% and 2.8% [70–72]. Populations of *An. harrisoni* and *An. aconitus* were found with very low sporozoite infections at 0.003% and 0.0006% of samples assayed, respectively (Table 3) [81].

Maculatus Group

Some members of the *An. maculatus* group are important vectors of malaria throughout the Oriental Region, including Thailand, Indonesia, Malaysia, and the Philippines [82]. Seven species are present in Thailand: *An. maculatus* Theobald, *An. sawadwongporni* Rattanarithikul and Green, *Anopheles dravidicus* Christophers, *Anopheles notanandai* Rattanarithikul and Green, *Anopheles willmori* James, *An. pseudowillmori* (Theobald), and *An. rampae* Harbach & Somboon [7,10,37,83,84]. *An. maculatus*, *An. pseudowillmori*, and *An. sawadwongporni* are regarded as important malaria vectors in Thailand [29,38]. *An. sawadwongporni* and *An. maculatus* are commonly encountered biting humans, often found in sympatry and in high density throughout Thailand, particularly along the border with Myanmar [10]. Most published studies (cited previously) on the biology and vector roles of these two species are based on morphological identification only.

From molecular-based identification, the behavior of *An. maculatus* and *An. sawadwongporni* are described from three locations in Thailand [56,57]. Both species exhibit zoophily and a tendency towards human feeding outdoors, with the exception of *An. sawadwongporni* in Mae Sot (Tak Province) showing stronger anthropophily [56]. *An. pseudowillmori* and *An. dravidicus* showed strong zoophilic and exophagic behaviors, except one population of *An. pseudowillmori* in northern Thailand with stronger anthropophily (Table 1) [56]. All four species of the Maculatus Group demonstrated greater biting activity between early evening and midnight [56,57]. Sporozoite infection rates of *An. maculatus*, *An. sawadwongporni*, and *An. pseudowillmori* have ranged between 0.1% and 3.1% (Table 2).

Molecularly identified *An. maculatus* and *An. sawadwongporni* from Vietnam and Myanmar have shown low sporozoite infection rates, ranging between 0.002% and 0.28% (Table 3). *Anopheles maculatus* is more likely to bite humans than *An. sawadwongporni*; however, in Vietnam, a population was observed to feed more on animals, with biting activity occurring predominantly during the first half of the evening [70,81].

Barbirostris Group

Within the Barbirostris Group, the Barbirostris Subgroup includes 11 species; ten have been formally named, with nine occurring in Thailand: *Anopheles donaldi* Reid, *Anopheles hodgkini* Reid, *Anopheles pollicaris* Reid, and six species of the Barbirostris Complex, *An. barbirostris* van der Wulp (former sp. A4, Clade I), *Anopheles campestris* Reid, *An. dissidens* Taai & Harbach (former

Table 3. Key Behavioral Traits and Infection of Malaria Vectors in the Greater Mekong Subregion[a]

Species	Location	Trophic tropism (or host preference)	Trophic behavior	Sporozoite infection rate (%)	Peak activity (hours)	Species identification methods (vector/parasite)	Refs
Anopheles dirus	Sekong, Lao PDR	Anthropophilic	Endophagic	0.55–0.81	21:00–24:00	PCR, ELISA	[44]
	Attapeu, Lao PDR	Anthropophilic	ND	0.17–2.69	22:00–01:00	PCR, ELISA	[43,67]
	Khammouan, Lao PDR	Anthropophilic	ND	ND	21:00–24:00	Phenotypic	[68]
	Quang Binh, Vietnam	Anthropophilic	Exophagic	ND	18:00–21:00	PCR-RFLP	[81]
	Binh Thuan, Vietnam	Anthropophilic	Endophagic	1.2	After 22:00	SCAR markers, ELISA	[70,71]
	Khanh Hoa, Vietnam	Anthropophilic	Exophagic	1.1	Before 22:00	SCAR markers, ELISA	[70,71]
	Rattanakiry, Cambodia	Anthropophilic	ND	10.7	After 22:00	SCAR markers, ELISA	[70,71]
	Myanmar			0.4–2.4	24:00–03:00	Phenotypic, ELISA	[69]
Anopheles baimaii	Myanmar	Anthropophilic	ND	6.25	ND	PCR, qPCR	[72]
Anopheles maculatus	Malaysia	ND	ND	10–15	ND	Phenotypic, ELISA	[126]
	Malaysia	ND	ND	0.02–1.24	ND	Phenotypic, ELISA	[127]
	Sekong, Lao PDR,	Anthropophilic	Exophagic	0.64–11.1	20:00–22:00	Phenotypic, ELISA	[44]
	Quang Binh, Vietnam	Zoophilic	Exophagic	0.002	18:00–21:00	PCR-RFLP, ELISA	[81]
	Hoa Binh, Vietnam	Zoophilic	Exophagic	ND	Before 22:00	Phenotypic, ELISA	[70,71]
	Binh Thuan, Vietnam	Zoophilic	Exophagic	ND	Before 22:00	Phenotypic, ELISA	[70,71]
	Khanh Hoa, Vietnam	Zoophilic	Exophagic	ND	Before 22:00	Phenotypic, ELISA	[70,71]
	Rattanakiry, Cambodia	Zoophilic	Exophagic	ND	Before 22:00	Phenotypic, ELISA	[70,71]
	Vientiane, Laos	Anthropophilic	Exophagic	ND	Before 22:00	Phenotypic, ELISA	[70,71]
	Myanmar	Anthropophilic	Exophagic	ND	22:00–24:00	PCR, qPCR	[72]
Anopheles sawadwongporni	Quang Binh, Vietnam,	Zoophilic	Exophagic	0.001	18:00–21:00	PCR-RFLP, ELISA	[81]
	Myanmar	Zoophilic	Exophagic	0.28	22:00–24:00	PCR, qPCR	[72]
Anopheles minimus	Sekong, Lao PDR,	Anthropophilic	Exophagic	ND	22:00 and 02:00	PCR	[44]
	Khammouan, Lao PDR	Zoophilic	Exophagic	ND	21:00–24:00	Phenotypic	[68]
	Quang Binh, Vietnam	Zoophilic	Exophagic	ND	ND	PCR-RFLP, ELISA	[81]
	Khanh Hoa, Vietnam	Anthropophilic	Endophagic	2.8	After 22:00	PCR-RFLP	[70,71]
	Rattanakiry, Cambodia	Zoophilic	Exophagic	1.4	ND	PCR-RFLP	[70,71]
	Vientiane, Laos,	Anthropophilic	Endophagic	0	After 22:00	PCR-RFLP	[70,71]
	Myanmar	Anthropophilic	Exophagic	1.4	01:00–02:00	PCR, qPCR	[72]
Anopheles harrisoni	Quang Binh, Vietnam	Zoophilic	Exophagic	0.003	18:00–21:00	PCR-RFLP, ELISA	[81]
Anopheles aconitus	Sekong, Lao PDR	Anthropophilic	Exophagic	ND	20:00–22:00	Phenotypic	[44]
	Quang Binh, Vietnam	Zoophilic	Exophagic	0.0006	20:00–22:00	PCR-RFLP, ELISA	[81]
	Rattanakiry, Cambodia	Zoophilic	Exophagic	ND	After 22:00	PCR-RFLP	[70]
	Vientiane, Laos	Anthropophilic	Exophagic	ND	Before 22:00	PCR-RFLP	[70]
Anopheles pampanai	Ninh Thuan, Vietnam	ND	ND	0.01	ND	Phenotypic, ELISA	[78]
	Binh Phuoc, Vietnam	ND	ND	20	ND	PCR, qPCR	[79]
Anopheles campestris	Bac Lieu, Vietnam	Anthropophilic	Endophagic	ND	After 22:00	Phenotypic	[70]
Anopheles sundaicus (= *An. epiroticus*)	Malaysia	ND	ND	0.4	ND	Phenotypic, ELISA	[126]

[a] ND, not determined/reported.

sp. A1, Clade III), *An. saeungae* Taai & Harbach (former sp. A2, Clade IV), *An. wejchoochotei* Taai & Harbach (former *An. campestris*-like, Clade V), and *An. barbirostris* sp. 3 [29,33,82,85–88]. Lastly, *Anopheles barbumbrosus* Strickland & Chowdhury within the Vanus Subgroup is also present in Thailand. *Anopheles barbirostris* is morphologically very similar to *An. campestris* but they have different host preferences, the latter species being closely associated with humans while the former is generally more zoophilic [87]. However, *Anopheles barbirostris* s.l. has been implicated as a natural vector of *Plasmodium* spp. and *Wuchereria bancrofti* in Sarawak State, Malaysia (Borneo Island) [6]. Coleman *et al*. [89] reported a mixed variant *P. vivax* sporozoite rate of 0.42% for *An. campestris* in Thailand. *Anopheles donaldi* is regarded as a secondary malaria vector in Malaysia; however, its presence in Thailand is believed to be too limited to serve as a vector of epidemiological concern [86]. The vast majority of studies describing the distribution, behavior, and other biological characteristics of *An. barbirostris* s.l. populations are based on phenotypic identification as no specific PCR assay for species identification was available until now [90]. In Cambodia, Laurent *et al*. [91] reported *P. falciparum* sporozoite infections in 0.25% (1/4) *An. barbirostris* and 0.04% (2/51) *An. dissidens* collected from cow-baited traps and molecularly identified using the universal primers of **internal transcribed spacer 2 (ITS2)**. However, these findings are subject to careful interpretation given the expanded number of cryptic species in the group known to present similar ITS2 length [33].

Sundaicus Complex

Of the four current members of this complex, only *Anopheles sundaicus* is regarded as an important malaria vector [46,92]. The complex is distributed widely from northeastern India and the Nicobar-Andaman Islands (India), eastwards to southern Vietnam, Malaysia, and Indonesia [92], primarily in coastal habitats. The four species comprise (i) *Anopheles epiroticus* Linton & Harbach (formerly species A), (ii) *An. sundaicus* s.s. (Rodenwaldt) (species B and C), (iii) *An. sundaicus* species D, and (iv) *An. sundaicus* species E [41,49,93]. Only *An. epiroticus* is present in Thailand (before 2005 it was reported in the literature as *An. sundaicus*) and restricted to eastern and southern coastal regions and islands [29,77]. Gould *et al*. [94] reported a greater exophagic frequency and a zoophilic preference for cattle in southeastern Thailand. As early as 1923, Barnes [25] reported oocysts (presumably *Plasmodium*) in *An. sundaicus* (= epiroticus) from Ko Phra Island, Chonburi Province (Gulf of Thailand).

Few published studies on the bionomics and behavior of the Sundaicus Complex have used molecular techniques for species identification. Cytogenetic and enzymatic techniques were initially used to identify putative species within the complex, wherein three forms of *An. sundaicus* were described, designated species A, B, and C from Indonesia [95]. Subsequently, these geographically separated (allopatric) species were studied using both cytochrome oxidase I (COI) and cytochrome b (Cyt-b), which allowed the development of an AS-PCR [49,92]. This assay revealed the presence of *An. sundaicus* s.s. in Sarawak and Kalimantan (Borneo Island), *An. sundaicus* species E in Sumatra and Java (Indonesia), and *An. epiroticus* in Cambodia, Thailand, and Vietnam [46,49,92]. *Anopheles sundaicus* species D appears restricted to the Andaman and Nicobar Archipelago (India), although it might also be present on the eastern coast of Sri Lanka [96]. Where studied in Thailand, *An. epiroticus* exhibits primarily anthropophilic and exophagic behavior (Table 1), whereas an island population showed stronger zoophily and even more pronounced exophagy [77], possibly reflecting differences in host availability by locality. In the 1960s, Vietnam recorded sporozoite infections in *An. epiroticus* ranging from 0.18% to 1.03% [97]. Forty years on, its role as a vector could not be confirmed due, in part, to the low malaria incidence in study areas [71]. In eastern Thailand, *An. epiroticus* is described as a secondary vector [98]. In southern Vietnam, *An. epiroticus* was reported to be resistant to several pyrethroids (permethrin, deltamethrin, α-cypermethrin, λ-cyhalothrin), whereas it has remained susceptible in Thailand [17,99].

Outdoor Malaria Transmission and Control Challenges

Mosquito behavioral factors (e.g., host preferences, activity patterns, feeding and resting locations, etc.) and physiological factors (vectorial capacity, longevity), seasonal climate variability [100], and susceptibility to insecticides [101] can influence the ability of a mosquito to transmit malaria parasites [102]. One aspect of transmission that has so far evaded effective and practical control measures is protection from outdoor biting vectors, so-called '**residual transmission**' [13,103,104]. This has become an increased concern as countries implement policies and programs to eliminate malaria transmission entirely [102]. Expanded vector surveillance and control tools targeting outdoor transmission are desperately needed, especially in Southeast Asia where many important vectors are primarily exophagic [105].

The two most widely accepted and used vector-control interventions are LLINs and IRS that primarily target vectors seeking human hosts indoors and/or rest inside homes. Depending on the active ingredient, the chemicals either function solely by direct killing action or may also have behavioral avoidance activity (e.g., contact locomotor excitation and spatial repellency) following sublethal exposure. Treated or untreated materials like netting can also function as a physical barrier to decrease vector–human contact. However, outdoor biting activity greatly limits the protective utility of LLINs and IRS, especially during early evening and morning hours when humans are outside and unprotected [105]. The effect of these two methods appears to have only modest if minimal impact on the outdoor-biting vector populations [13]. To what extent outdoor transmission is important in the larger epidemiological and control context will vary depending on vector species' behavior and local epidemiological conditions [106]. Therefore, well designed investigations are required in areas of risk to determine the correct target vector species and a practical spatial and temporal balance of control tools to deploy.

Thailand's national malaria control program uses various vector-control methods to interrupt indoor transmission [107], yet, as in other areas in the GMS, outdoor transmission confounds effective control efforts [13]. Chemicals with sublethal excito-repellency actions that cause behavioral avoidance in vectors deserve greater attention regarding impact on operational control and transmission reduction [108]. Speculation exists that chemicals used to curb indoor transmission may increase, by way of behavioral diversion, a selective advantage to mosquitoes that feed and rest outdoors, thus promoting increased relative risk of transmission occurring outdoors [103,109]. On the other hand, indoor control methods may have a broader impact ('area effect') beyond the interior treated household extending into the surrounding community [110]. The influence of compounds possessing excito-repellent properties on malaria transmission will depend on site-specific behavioral characteristics of vector populations. For example, most *An. dirus* populations exhibit a very strong exophagic/exophilic behavior, whereas *An. minimus* and *An. maculatus* populations appear more opportunistic in their feeding and resting habits [10,76,111]. Therefore, generalizations on the importance of outdoor transmission are inappropriate; rather, site-specific investigations are required to determine the actual transmission dynamics and the relative importance of outdoor exposure. Moreover, depending on the strategic goal (e.g., control versus elimination), the relative contribution of outdoor transmission to overall malaria incidence must be determined, prioritized, and factored in for intervention.

A combination of established and newer vector control tools should be evaluated to target more precisely those mosquitoes with a greater proclivity to feed outdoors and during early evening hours [112]. Additionally, personal protective devices, such as clothing and topical repellents, can provide added protection against indoor and outdoor biting attacks [113]. Existing outdoor adult mosquito-control methods include insecticidal space spray ('fogging' or ultra-low volume applications) and larval habitat source management; both are capable of reducing vector densities to varying degrees and duration. Other methods have shown promise and/or are under development (e.g., insecticide-treated cattle, male swarm spraying, 'push–pull system' using combinations of spatial repellents and attractant traps, sterile insect techniques, and endectocides) as possible vector-control tools under the correct circumstances [105,112,114]. Other innovations, such as insecticide-treated 'eave tubes' and attractive toxic sugar bait systems (ATSBs) use a strategy of 'attract and kill' that exploit the innate behavior of mosquitoes; both methods, for example, allow contact with a lethal insecticide by males and females that naturally feed on sugar sources, or females that are attracted to host cues [115,116]. More studies and epidemiological field trials are required using eaves tubes, ATSBs, barrier screening, and other innovative methods for vector control that may prove effective under the correct circumstances, while being target-specific with minimal contamination of the environment.

In order to attain malaria elimination, concerted actions by national malaria-control programs, with the support of regional partners, should strengthen public health entomological capacity, particularly in advanced techniques for the accurate identification of mosquitoes using molecular methods. Without a better understanding of mosquito vectors, linking precise species identification with important bionomic and behavioral traits that promote transmission, achieving malaria elimination without a more comprehensive approach to vector control will be a more arduous undertaking.

Concluding Remarks

This review selected published laboratory and field studies on malaria vectors, over a 30-year period (1983 to 2019), described in 16 studies from Thailand and 15 other investigations in neighboring GMS countries. Current knowledge is highlighted, while many gaps in understanding remain regarding mosquito bionomics, for example, feeding behavior. With renewed emphasis on vector control and the scale-up of activities in many malaria-endemic countries worldwide, there has been an overall reduction in malaria morbidity and mortality. However, reduction in the risk of disease transmission has varied across and within countries for many reasons. Significant differences in transmission intensity can naturally exist between locations, compounded by varying degrees of outdoor malaria transmission that escape the full impact of common interventions like LLINs and IRS.

The current gaps in knowledge surrounding outdoor transmission include: (i) prevalence and distribution of malaria infection to identify the magnitude of outdoor-acquired infection, (ii) vector population abundance, composition, and behavior, (iii) mosquito–human contact patterns and routes of transmission, and (iv) defining human behavior contributing to exposure risk (see Outstanding Questions). All of these aspects require more accurate and timely information to combat this neglected aspect of malaria transmission epidemiology. A better understanding of the spatial and temporal heterogeneity in malaria transmission and vector species is critical for identifying and implementing targeted and more effective vector-control strategies. The accurate identification of vector species is a critical component of that understanding.

The existence of *Anopheles* species complexes generates additional difficulties in accurately identifying different members of sibling species by morphological means alone. The ability to separate and distinguish each sibling species is of epidemiological and operational importance as each species can differ significantly from one another in biological characteristics. Examples include variations in preferred microhabitats, adult resting sites, host preferences, biting behaviors, longevity, susceptibility to insecticides, and susceptibility (competence) to malaria parasites, all of which contribute to a species’ importance and capacity for transmitting pathogens. Moreover, heterogeneous behavioral traits can occur across a species geographical range, likely influenced by, and a consequence of, local environmental conditions and other circumstances (e.g., host availability and behavior). Incorrect identification between sibling species, in particular distinguishing between vector and nonvector species, can lead to inefficient use of time and resources in vector control [117]. Unless the vector ‘target’ is clearly identified, control efforts can become wasteful, inefficient, and misdirected [11,118,119]

Outstanding Questions

What is the next chapter for effective vector control?

Why has the burden of malaria not decreased in certain malaria areas despite the implementation of multiple control measures?

Is outdoor vector control needed for malaria elimination?

How will the changing land use and human activities impact malaria transmission?

Which alternative controls should be suggested in areas where residual transmission occurs?

Acknowledgments

We are grateful to the anonymous reviewers for providing useful guidance to improve the manuscript. We thank Dr Wanapa Ritthison for Thailand malaria case data. Special thanks to Brian Bacay, for constructive comments on the initial draft manuscript. This study was supported by the International Research Network (IRN58W0003), Thailand Research Fund (TRF), Thailand, the JEAI-VECTHAI (Jeune Equipe Associée IRD) funding provided by the French National Research Institute for Sustainable Development (IRD France), Montpellier, and a PhD fellowship (ARTS) by IRD provided to the senior author.

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