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ANOPHELES (DIPTERA: CULICIDAE) SPECIES COMPLEX IN THAILAND: IDENTIFICATION, DISTRIBUTION, BIONOMICS AND MALARIA-VECTOR IMPORTANCE

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Abstract- *Anopheles* mosquitoes are still considered to be important vectors worldwide, with approximately 80 species of them incriminated as vectors of malaria, filarial nematode and encephalitis virus. Among these, at least 30 species exhibit species complexes, which comprise about 145 sibling species members. The exhibition of species complexes within the taxon of some *Anopheles* lead to complication of vector control that results from the difficulty in precisely identifying sibling (isomorphic) species members and their difference in biological characteristics. During the past 3 decades in Thailand, at least 6 malaria vectors, i.e., *An. dirus*, *An. minimus*, *An. maculatus*, *An. sundaicus*, *An. barbirostris* and *An. leucosphyrus* were proven to be species complexes. Thus, the objective of this review is to provide the current taxonomical information of Thai *Anopheles* species complexes, including techniques used in the identification of sibling species members, their geographic distribution, bionomic status and malaria-vector importance.
Key words- *Anopheles*, species complex, identification, distribution, trophic behavior, malaria vector, Thailand.

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Introduction

Five malaria species (*Plasmodium vivax*, *P. falciparum*, *P. malariae*, *P. ovale* and *P. knowlesi*) transmitted by *Anopheles* mosquitoes are still a major public health problem in the world. At least 109 countries, comprising 8 parts (Africa, Asia, the Middle East, Eastern Europe, Central and South America, Hispaniola and Oceania) of tropical and subtropical regions are endemic areas of these parasites, and threaten the health of about 250 million people per year [1]. Recently, *P. knowlesi*, a primate malaria parasite in macaques, was first reported to infect humans in the Malaysian peninsular [2]. In Thailand, the most common malaria species are *P. vivax* and *P. falciparum*, whereas *P. malariae* and *P. ovale* are rare [3-4]. However, a total of 35 cases of *P. knowlesi* were reported recently from 6 provinces, comprising 3 regions in Thailand, i.e., western (Tak and Prachuap Khiri Khan provinces), eastern (Chanthaburi province) and southern (Ranong, Yala and Narathiwat provinces) [5-8]. Regarding *P. ovale*, based on DNA samples from Ghana, Myanmar, Nigeria, Sao Tome, Sierra Leone and Uganda, at least two distinct new species, i.e., *P. ovale curtisi* (classic type) and *P. ovale wallikeri* (variant type), have been described recently [9]. Nonetheless, the real identity of these two new malaria species in Thailand is still a crucial question, which requires further intensive investigation. The disease is limited generally to rural communities living in and near forested areas, mountains and foothills, particularly those residing in newly opened land settlements of semi forested areas and earning their living by growing agricultural crops as well as those living in areas near and along the borders with neighboring Cambodia, Laos, Myanmar and Malaysia [3-4].

At least 21 *Anopheles* species are reported in Thailand as primary (regional), secondary (local) and suspected vectors of malaria. The primary vectors are *An. dirus*, *An. baimaii*, *An. minimus* and *An. maculatus*, while *An. aconitus*, *An. pseudowillmori* and *An. epiroticus* are considered as secondary vectors, based on the recovery of sporozoites from salivary glands and their geographic distribution [10-18]. The remaining 14 species, i.e., *An. annularis*, *An. barbirostris*, *An. campestris*, *An. hodgkini*, *An. karwari*, *An. kochi*, *An. nigerrimus*, *An. nivipes*, *An. peditaeniatus*, *An. philip-*

pinensis, *An. sawadwongporni*, *An. sinensis*, *An. tessellatus* and *An. vagus* are suspected vectors, based on positive oocysts in the midgut and/or enzyme linked immunosorbent assay of circumsporozoite antigens [11, 13, 15, 19-26]. *An. barbirostris*/*campestris* group are considered as potential vectors that play an important role in the increasing cases of *P. vivax* infection in Thailand, based on their anthropophillic behavior, and high oocyst and sporozoite rates from laboratory susceptibility tests [27-29, 111]. *An. latens* and *An. cracens*, have been incriminated recently as natural vectors of *P. knowlesi* in the Malaysian peninsular [30-31], and are thus, considered provisionally as possible vectors that might play a role in transmitting this malaria parasite in the southern region, based on their distribution in southern Thailand.

Although vector control programs have been established in Thailand for a long time, the diseases continue to be endemic year by year. The partial failure to control vectors has many components, e.g., no and/or incomplete insecticide spraying in the household, change in vector biting habits, vector tolerance or resistance to insecticides and its exhibition of species complexes [32-34]. The last factor appears to be important and presumably affects all other aspects, since it leads to difficulty in precisely identifying sibling species members that possess identical morphology or minimal morphological distinction. In addition, those members may differ in biological characteristics (e.g., microhabitats, resting and biting behavior, sensitivity or resistance to insecticides, susceptible or refractory to malaria parasites, etc.), which can be used to determine their potential for transmitting diseases. Incorrect identification of individual members in *Anopheles* species complexes may result in failure to distinguish between a vector and non-vector, and lead to complications and/or unsuccessful vector control [34-35].

Throughout the world, a total of 478 species of *Anopheles* mosquitoes have been discovered, and approximately 80 of them play an important role as vectors of malaria, filarial nematode and encephalitis virus. Among these, at least 30 species exhibit species complexes, which comprise about 145 sibling species members [34-37]. In Thailand, significant progress has been made in the population genetic study of primary vectors: *An. dirus* [38-43], *An. minimus* [44-51] and *An. maculatus* [52-58]; secondary vectors: *An. aconitus* [59], *An. maculatus* (species I/*pseudowillmori*) [60] and *An. sundaicus* [16, 61-63]; suspected vectors: *An. maculatus* (species A/*sawadwongporni*) [64], *An. sinensis* [65], *An. peditaeniatus* [66] and *An. vagus* [67]; potential vectors: *An. barbirostris*/campestris group [68-71]; and possible vector: *An. leucosphyrus* [72]. However, only 6 species, i.e., *An. dirus*, *An. minimus*, *An. maculatus*, *An. sundaicus*, *An. barbirostris* and *An. leucosphyrus*, exhibited species complexes.

Techniques used in the identification of sibling species members

So far, at least 1 or 2 traditional techniques have been used widely for the recognition of sibling species within the taxon *Anopheles* species complex at post- and pre-mating barriers. For post-mating barriers; the hybridization or crossing experiment, using the artificial mating technique to determine hybrid non-viability, sterility or breakdown, is still a useful tool for identifying sibling species members of each complex. Detailed genetic incompatibility, including lack of insemination, embryonation, hatchability, larval survival, pupation, emergence, adult sex distortion, abnormal reproductive system and complete or incomplete (only at the inversion heterozygote regions in some cases) asynaptic salivary gland polytene chromosomes are useful criteria for elucidating species complex status. However, a point worth noting is that an iso-female line (isoline) colony established from the combinative characters of morphological and/or cytological markers has to be considered seriously. A laboratory raised colony established from a naturally mixed population should be omitted, since it may be a mixture of cryptic species [34, 38-39, 41, 73]. As for pre-mating barriers; examination of the polytene chromosomes in wild-caught adult females, and/or progenies of isolines, provides clear evidence that different specific mate recognition systems (SMRS) exist. The total absence or significantly deficient number of heterozygotes for an inversion in a population entirely indicates the presence of reproductive isolation within a taxon [34, 54, 59, 74]. Nonetheless, at least 3 problems have been raised regarding this matter, i.e., (1) a skilled person is needed to prepare a perfect chromosome and make an identification, (2) homosequential banding species cannot be employed, e.g., *An. maculipennis* complex [75] and *An. barbirostris* complex [68-71], and (3) a relatively large amount of sample materials are required to perform the Hardy-Weinberg equilibrium, which cannot be applied to small numbers of rare species specimens that are caught during specific seasons. Electrophoretic variations at enzyme loci are not only useful for identification of sibling species, but also for the correct identification of morphologically cryptic *Anopheles* species. Variations at a locus thus enable detection of reproductive isolation within populations, resulting from positive assortative (preferential) mating [34, 47, 74]. Nevertheless, at least 2 problems have been raised regarding this technique, i.e., (1) specimens must be fresh or frozen until analysis, and (2) its use must be similar to that of the polytene chromosome, as it requires a relatively large amount of sample materials to perform the Hardy-Weinberg equilibrium, as previously described.

In light of the advantages and disadvantages of the techniques mentioned above, the rapid systematic procedures for the identification of *Anopheles* species complexes were formed recently [76]. This was done by crossing experiments among iso-female lines using karyotypic markers (characteristics of metaphase karyotypes/karyotypic forms) related to DNA markers [comparative DNA sequence analyses of some specific genomic loci (e.g., ribosomal DNA: ITS2; mitochondrial DNA: COI, COII) that determined large sequence divergence or very low intraspecific sequence variation] of each isoline colony. By applying of this rapid systematic procedure, 5 sibling species members i.e., *An. barbirostris* species A1, A2, A3 and A4, and *An. campestris*-like, have been identified in the taxon *An. barbirsotris* within the last 2 years [68-71]. Remarkably, rare allopatric species with few isolines such as *An. barbirostris* species A3 and A4, which could be caught in a specific season and/or locality, continue to perform genetic proximities among other sibling species members. In addition, the 5 sibling species members of *An. barbirsotris* complex exhibited homosequential banding patterns of salivary gland polytene chromosomes, with a marked limitation of use to investigate their pre-mating barriers by determining inverted heterozygote in a natural sympatric population.

Geographic distribution, bionomics and malaria-vector importance

Regarding the difference in geographic distribution, trophic behavior, vector competence and malaria-vector importance among sibling species members within the taxon *Anopheles* vectors, great progress or positive findings have been recovered in the studies of *An. dirus* complex, *An. minimus* complex, *An. maculatus* complex, *An. sundiacus* complex, *An. baribrostris* complex and *An. leucosphyrus* complex. Summarization of geographic distribution is shown in Fig. 1, known sibling species members related to malaria-vector importance are demonstrated in Table 1, and details are as follows:

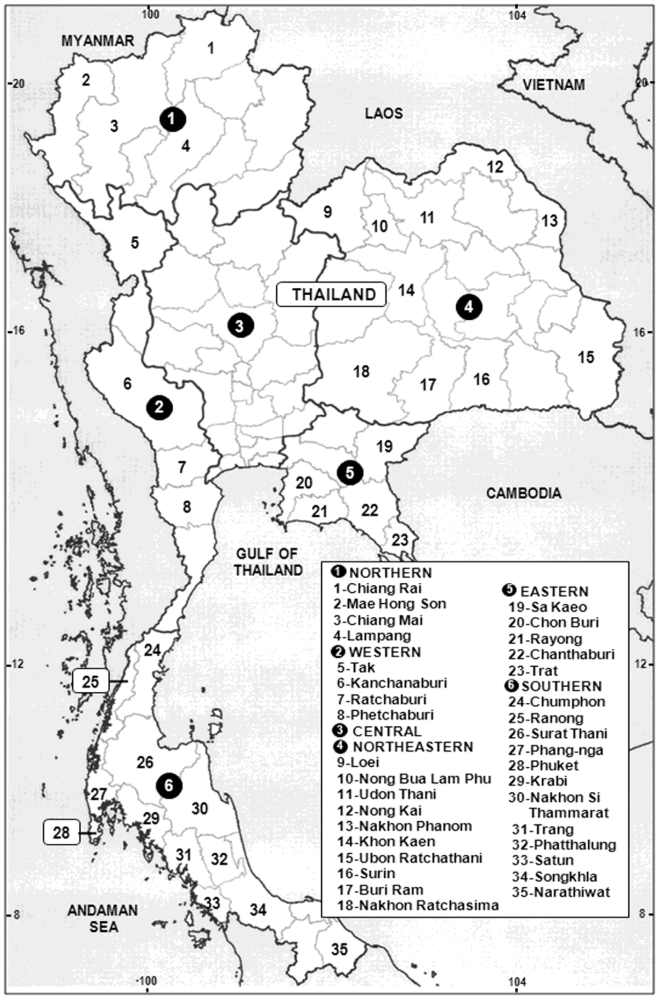

Fig. 1- Map of Thailand, showing distribution of the species members of 6 *Anopheles* species complexes. Cosmopolitan distribution across 6 regions: *An. barbirostris* species A1, *An. campestris*-like, *An. dirus*, *An. maculatus* and *An. minimus*. Distribution in some specific provinces: *An. baimaii* (5, 6, 25, 27, 29); *An. barbirostris* species A2 (4, 7, 8, 11, 15, 22), A3 (6) and A4 (3); *An. cracens* (27, 29, 31, 32, 34); *An. dravidicus* (1, 2, 3, 5, 6, 9, 18); *An. epiroticus* (21, 22, 23, 24, 27, 28, 29, 30, 31, 33, 34); *An. harrisoni* (3, 5, 6); *An. latens* (30, 35); *An. maculatus* Form E (24, 34); *An. nemophilous* (5, 6, 25, 27, 30, 34); *An. notanandai* (7, 8); *An. pseudowillmori* (1, 2, 3, 5, 9, 12); *An. rampae* (10, 12, 13, 15); *An. sawadwongporni* (18, 25, 27); *An. scanloni* (6, 25, 27, 29, 30, 31, 32); and *An. willmori* (3).

Table 1- Known sibling species members of Anopheles species complexes as primary, secondary, suspected, potential and possible malaria-vectors in Thailand and neighboring countries

Species complexes/ sibling species members	Human malaria (*P. vivax, P. falciparum, P. malariae and P. ovale*)		Macaque malaria (*P. knowlesi*)	
	Vector in Thailand	Vector in neighboring countries	Vector in Thailand	Vector in neighboring countries
An. dirus complex				
An. dirus	1	+	-	-
An. baimaii	1	-	-	-
An. cracens	-	-	5	+
An. minimus complex				
An. minimus	1	+	-	-
An. maculatus complex				
An. maculatus	1	+	-	-
An. pseudowillmori	2	-	-	-
An. sawadwongporni	3	-	-	-
An. sundaicus complex				
An. epiroticus	2	+	-	-
An. barbirostris complex				
An. campestris-like	4	-	-	-
An. leucosphyrus complex				
An. latens	-	+	5	+

1- *primary vector,* 2- *secondary vector,* 3- *suspected vector,* 4- *potential vector,* 5- *possible vector*

***An. dirus* complex-** Morphological variations observed in natural populations, biological and behavioral difference of laboratory bred colonies (stenogamy or eurygamy), cytological [metaphase karyotypes and salivary gland polytene chromosomes: differences in banding patterns at the free ends of 1 (X), 2 (2R) and 3 (2L)] analyses of laboratory colonized and natural populations, and crossing experiments among isoline colonies have led to the recognition of 7 sibling species members of this complex, i.e., *An. dirus* (*dirus* A), *An. cracens* (*dirus* B/*balabacensis* Perlis Form), *An. scanloni* (*dirus* C), *An. baimaii* (*dirus* D), *An. elegans* (*dirus* E), *An. nemophilous* (*dirus* F) and *An. takasagoensis* [39, 41, 73, 77-82] In Thailand, only five sibling species members, i.e., *An. dirus*, *An. cracens*, *An. scanloni*, *An. baimaii* and *An. nemophilous* are found indigenously [83]. *An. dirus* is a cosmopolitan species that distributes across 6 regions (northern, southern, central, northeastern, eastern and southern) in Thailand, while *An. baimaii*, *An. cracens*, *An. nemophilous* and *An. scanloni* distribute in sympatric with *An. dirus* in western and/or southern regions. Detailed species distributions together with regions and/or provinces are illustrated in Fig. 1.

Observation on the biting activity of *An. baimaii*, *An. cracens*, *An. dirus* and *An. scanloni* revealed that these four isomorphic species feed at different times during the night [84]. The case of early biting in *An. scanloni* at Nakhon Si Thammarat province, southern Thailand, is strikingly different from cases in the other species, although all of them are anthropophilic. Outdoor biting activity of *An. scanloni* is normally at a high level in early evening, at around 18:00-20:00 h. It then declines sharply and is maintained at a very low level throughout the second half of the night. *An. cracens* at Phatthalung province, southern Thailand, exhibited a slightly different feeding activity from *An. scanloni*, with a peak period of outdoor biting time at around 19:00-21:00 h, and a low level main-

tained throughout the second half of the night. Interestingly, in 20 years, this biting peak has been mostly in agreement with the biting activity of *An. cracens* (peak period: 19:00-20:00 h) strain from the State of Pahang, Malaysia, which is adjacent to southern Thailand [31]. In contrast, the outdoor biting activity of *An. dirus* at Phitsanulok province, central Thailand, started somewhat later in the first half of the night, with a peak period at around 21:00-23:00 h. The outdoor biting activity of *An. baimaii* at Krabi province, southern Thailand, was even later than the others, beginning at a low level and gradually increasing to a peak period in the second half of the night at around 01:00-03:00 h. Among these, *An. dirus* and *An. baimaii* were incriminated as primary vectors of *P. vivax* and *P. falciparum* [13-14, 83], while *An. cracens* was a possible natural vector of *P. knowlesi* in southern Thailand, based on its distribution there, and also incriminated as a natural vector of *P. knowlesi* in Kuala Lipis District in the State of Pahang, Malaysia [31] (Table 1).

***An. minimus* complex-** Three sibling species members were recovered within the taxon *An. minimus* complex, i.e., *An. minimus* (*minimus* A), *An. harrisoni* (*minimus* C) and *An. yaeyamaensis* (*minimus* E). Morphological (M, V and P forms) and isoenzyme (esterase and octanol dehydrogenase) studies together have recognized *An. minimus* and *An. harrisoni* in a sympatric population [44, 47], while *An. yaeyamaensis* was discovered by crossing experiments relating to comparative morphological, cytological (metaphase karyotypes) and DNA (D3 region) investigations with *An. minimus* and *An. harrisoni* [85-87]. *An. minimus* is the predominant species of the complex and distributes across 6 regions in Thailand, and also in the Oriental region (India, Vietnam, China and Taiwan) [34, 47, 88-91] *An. harrisoni* was recorded commonly in Kanchanaburi province, central Thailand, and found in sympatric with *An. minimus*, but absent or rare in other provinces [44, 47, 49] (Fig. 1). Based on enzyme electrophoresis, *An. harrisoni* has been reported in Vietnam [90], where it occurs in sympatric with *An. minimus* in varying proportions depending upon locality, host preferences and season. *An. yaeyamaensis* has so far been reported from only islands of the Ryukyu Archipelago, Japan [85, 87].

Observation on the biting activities of *An. minimus* and *An. harrisoni* at Ban Phu Toei, Sai Yok district, Kanchanaburi province, western Thailand revealed that *An. minimus* bit on humans more than animals, while *An. harrisoni* bit mainly on animals [44]. These results were contrary to subsequent studies, although the same area of interest was investigated [92]. The results revealed that both *An. minimus* and *An. harrisoni* tended to feed from cows rather than humans, and they did not find any preference for indoor-, outdoor- or forest-biting in either species. Both species had a peak biting density in October/November, at the end of the rainy season. Additional studies by other investigators in western Thailand indicated that *An. minimus* is mainly anthropophilic, endophagic and exophilic, while *An. harrisoni* has shown a greater tendency of zoophily, exophagy and exophily. *An. harrisoni* exibits two peaks of biting activity, the first in the early evening, between 18:00-21:00 h, with a second small peak from midnight to 02:00 h or from 03:00-06:00 h, whereas *An. minimus* tends to bite later, with peak activity occurring around 22:00 h [22, 93-94]. *An. minimus* was incriminated as a primary vector of malaria in Thailand [12, 14-15], whereas the vector status of *An. harrisoni* for transmitting malaria in nature has not been determined up to this time (Table 1). However, reports on laboratory susceptibility to *P. vivax* and *P. falciparum* of these 2 anopheline species indicated that *An. minimus* and *An. dirus* yielded rather similar susceptibility rates to both in *P. vivax* [oocyst rates (8 days post-infection): *minimus* = 81.82, *dirus* = 100; sporozoite rates (12 days post-infection): *minimus* = 77.78, *dirus* = 100] and *P. falciparum* [oocyst rates (8 days post-infection): *minimus* = 91.67, *dirus* = 100; sporozoite rates (12 days post-infection): *minimus* = 100, *dirus* = 95.65], whereas *An. minimus* yielded higher susceptibility rates to *P. vivax* than *An. harrisoni* [oocyst rates (8 days post-infection): *minimus* = 81.82, *An. harrisoni* = 50.00; sporozoite rates (12 days post-infection): *minimus* = 77.78, *An. harrisoni* = 52.17], although they had no statistically significant difference [59].

***An. maculatus* complex-** Comparative morphological (reduced abdominal scaling, heavy abdominal scaling and non-scaly forms) and cytological [metaphase chromosomes and ovarian nurse cell polytene chromosomes: 1 (X) and 2 (2R)] studies together have identified 8 sibling species members in this complex [*An. sawadwongporni* (species A), *An. maculatus* (species B plus metaphase karyotype Form E), *An. dravidicus* (species C), *An. notanandai* (species G), *An. willmori* (species H), *An. pseudowillmori* (species I), *An. greeni* (species D) and *An. dispar* (species J)] [55-56, 83, 95-99]. Recently, *An. rampae* (*maculatus* metaphase karyotype Form K) was identified by crossing experiments relating to comparative morphological, cytological (metaphase and polytene chromosomes) and molecular (rDNA: ITS2, D3; mtDNA: COII, ND5) investigations with other species members [100-104]. In Thailand, seven species members, i.e., *An. sawadwongporni*, *An. maculatus* (plus Form E), *An. dravidicus*, *An. notanandai*, *An. willmori*, *An. pseudowillmori* and *An. rampae* are found, while *An. greeni* and *An. dispar* are indigenous to the Philippines [34, 83, 104-105]. Regarding distribution of the species members of *An. maculatus* complex in Thailand, *An. maculatus* is a cosmopolitan species that distributes across 6 regions in Thailand, but *An. maculatus* Form E is limited to only the southern region. *An. dravidicus* and *An. pseudowillmori* are found sympatric with *An. maculatus* in northern, western and northeastern regions. *An. sawadwongporni* is recorded in northeastern and southern regions, whereas *An. willmori* and *An. notanandai* are confined only to northern and western regions, respectively (Fig. 1).

Biting activities of the species members of *An. maculatus* complex in Thailand were studied in Pakchong district, Nakhon Ratchasima province, central Thailand and Sadao district, Songkhla province, southern Thailand [106]. In Pakchong district, *An. sawadwongporni* was the most dominant species, followed by *An. maculatus* and *An. dravidicus*, which were rare. The densities of *An. sawadwongporni* and *An. maculatus* were high between July and November, with their peaks in October. Biting activities of both species occurred throughout the night, with a major peak during the first quarter of the night in all seasons. Similar result of peak duration between 18:00-20:00 h was obtained from the bites of *An. maculatus* in Tak province, western Thailand [22]. In Sadao district, only *An. maculatus* (plus Form E) was obtained with peak densities between February and June. Biting activities of this species varied according to the season. All species identified in the

study were found to be predominantly zoophagic and preferred to bite humans outdoors, rather than indoors. Apart from these, *An. maculatus* and *An. pseudowillmori* have been incriminated as primary and secondary vectors of malaria in southern and western region, respectively, while *An. sawadwongporni* is considered as a suspected malaria-vector [14, 55, 83] (Table 1).

***An. sundaicus* complex-** At least 5 sibling species members, i.e., *An. epiroticus* (*sundaicus* A), *An. sundaicus* s.s. (*sundaicus* B and C), *An. sundaicus* D and *An. sundaicus* E were discovered within this complex, and only *An. epiroticus* was found indigenously in Thailand [11, 83]. *An. sundaicus* A, B and C were recognized based on the distinct characteristics of metaphase karyotypes (Form A, B and C) together with ovarian nurse cell polytene chromosomes [distinct banding patterns at the tip of chromosome 1 (X) and at the proximal region of chromosome arm 2 (2R)] [62]. Additional evidence to ascertain their biological species resulted from positive assortative mating for 12 enzyme-electromorph loci and phylogenetic dendrogram mixtures of *An. sundaicus* A, B and C were created [63]. The evidence to support *An. sundaicus* D is the discovery of a new cytogenetic variant (cytotype D), which was raised from the combinative characteristics of the ovarian nurse cell polytene chromosome of *An. sundaicus* A and C (Xa and 2Rb chromosomal-typed) [107]. Molecular identification using ITS2 and D3 regions, which could separate *An. sundaicus* D from *An. sundaicus* A, B and C, formed strong supportive evidence [108]. Based on the 2.1% mean sequence variation in both COI and cytochrome b (Cyt-b) genes of mtDNA among *An. sundaicus* A, B and C, the formerly named *An. sundaicus* A was proposed as *An. epiroticus* [16]. Additionally, phylogeography investigation of *An. sundaicus* s.l. collected from Indonesia, based on COI and Cyt-b sequences, revealed a distinct species, which was designated as *An. sundaicus* E [109]. Subsequently, an allele specific PCR was developed for distinguishing among *An. epiroticus*, *An. sundaicus* B and C, and *An. sundaicus* E [110].

An. sundaicus s.l. is considered as a vector of malaria in coastal areas of some countries, and is distributed widely in Oriental regions, extending from India, east to China through Bangladesh, Myanmar, Indochina, Thailand, Malaysia, Singapore and Indonesia [11, 83, 111-112]. It is generally a brackish water breeder, but *An. epiroticus* from south Tapanuli, north Sumatra, Indonesia [62-63], and *An. sundaicus* D from Teressa, Nancowry, Car Nicobar and Katchal islands, India [108], are freshwater breeders. *An. sundaicus* E is found to be restricted in Sumatra and Java, Indonesia [109]. In Thailand, only *An. epiroticus* has been recorded and is widespread along the coastal areas of eastern and southern regions [11, 62-63, 83]. (Fig. 1).

Adult females of *An. sundaicus* s.l. rest by day both indoors and outdoors, and are attracted more to cattle than humans but readily bite the latters indoors [11, 111]. Recently, the bionomic status of *An. epiroticus* has been performed intensively in Rayong province, eastern Thailand. The biting pattern increased during 18:00-20:00 h and maximized at midnight (21:00-24:00 h). A total of 926 wild-caught female *An. epiroticus* was investigated for *P. falciparum* and *P. vivax* by using Nested PCR and real-time PCR techniques. The results revealed that 3 and 6 specimens were positive for *P. falciparum* and *P. vivax*, respectively. In addition, the overall annual entomological inoculation rate (EIR) and parity rate of this species was 76.6 and 74, respectively [113]. These results confirmed the secondary vector status of *An. epiroticus* in coastal locations of Thailand, as in former reports [11] (Table 1).

***An. barbirostris* complex-** Five sibling species members, i.e., *An. barbirostris* species A1, A2, A3 and A4 and *An. campestris*-like, were recognized recently within the taxon *An. barbirostris* complex by means of crossing experiments among isolines relating to morphological characters (summation of seta 2-VI of pupal skins), cytogenetic forms (metaphase karyotypes) and DNA sequence analysis of ribosomal DNA (ITS2) and mitochondrial DNA (COI, COII) [68-71].

Observation on biting behavior of wild females indicated that *An. barbirostris* species A1, A2, A3 and A4 are zoophily, exophagy and exophily, whereas *An. campestris*-like is anthropophilic, exophagic and exophilic [68-71]. Studies on distributional characteristics of the species members of *An. barbirostris* complex revealed that *An. barbirostris* species A1, A2, A3 and A4 are forested-mountainous anophelines, while *An. campestris*-like is a plain-location species. *An. barbirostris* species A1 is distributed widely across 6 regions in Thailand, whereas *An. barbirostris* species A2 occurs sympatrically with *An. barbirostris* species A1 in some populations of northern, western, northeastern and eastern regions. *An. barbirostris* species A3 and A4 were confined to western and northern regions, respectively. *An. campestris*-like was a cosmopolitan species in plain localities throughout Thailand (Fig. 1).

The potential vector status of 5 species members of *An. barbirostris* complex for malaria parasites was investigated by artificial membrane feeding on blood containing gametocytes of *P. falciparum* and *P. vivax*, and dissected for oocyst and sporozoite rates 8 and 14 days post blood-meal [71]. The total non-development of oocysts and sporozoites from *An. campestris*-like (Chiang Mai and Udon Thani strains), and *An. barbirostris* species A3 and A4 indicated that these anophelines were entirely refractory vectors for *P. falciparum*. The low normal development of oocysts (oocyst rates: 40-60) and sporozoites (sporozoite rates 6.67-11.76) recovered from *An. barbirostris* species A1, A2 and A3, demonstrated their low potential vectors for *P. vivax*. The high normal development of oocysts (oocyst rates: 100) and sporozoites (sporozoite rates: 64.29-66.67) obtained from *An. campestris*-like (Chiang Mai strain) indicated their high potential vectors for *P. vivax*. The present results confirmed the potential vector status of the *An. barbirostris*/*campestris* group in transmitting *P. vivax* in Thailand [27-29] (Table 1). Additionally, this information asserted the previous proposed that different sibling species members within the complexes may result in the difference in malarial vector-competence, and lead to the complication of vector control-approaches [34].

***An. leucosphyrus* complex-** So far, 2 sibling species member, i.e., *An. latens* (*leucosphyrus* A) and *An. leucosphyrus* (*leucosphyrus* B) have been discovered within this taxon. *An. latens* was distributed in southern Thailand (Fig. 1), the Malaysian peninsular and Kalimantan, while *An. leucosphyrus* was found mainly in Sumatra, Indonesia [72, 111]. Observation on the biting activity of *An. latens* in Kapit district, Sarawak, Malaysian Borneo indicated that it started biting from 18:00 h, with a peak biting time between 19:00 and 20:00 h in the forest, as opposed to between

01:00 and 02:00 h on the farm [114]. *An. latens* (formerly as *An. leucosphyrus* s.l.) has been incriminated as a vector of human malaria in Sarawak and Sumatra [111], and it was reported recently as a natural transmitted-vector of *P. knowlesi* from macaques to humans in Kapit District, Sarawak, Malaysian Borneo [30]. It was also thought to be a possible vector in transmitting *P. knowlesi* to humans in southern region [5-8] (Table 1).

Conclusion

The difference in geographic distribution, trophic behavior, vector competence and malaria-vector importance among sibling species members within the taxon *An. dirus* complex, *An. minimus* complex, *An. maculatus* complex, *An. sundaicus* complex, *An. barbirostris* complex and *An. leucosphyrus* complex in Thailand, as in the detailed information above, indicates the significance of control approaches. All outstanding information is necessary for intensive evaluation and proper selection in forming a low cost and highly effective control strategy in order to interrupt the transmission of malaria parasites by these *Anopheles* vectors at specific locations and/or comparatively wide-range geography.

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