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## **Anopheles** species diversity and distribution of the malaria vectors of Thailand

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In Thailand, seven *Anopheles* species within three species assemblages have been incriminated as important malaria vectors. Comprehensive maps showing the countrywide geographical distribution of primary and secondary malaria vector species have not yet been developed; the maps that do exist are typically restricted to specific areas or are out of date. In addition, with the advent of molecular-based species-identification tools, the geographical locations of various sibling species have been more clearly defined in the country. This review has compiled published data on the known distribution of important malaria vectors in Thailand based on genetic and molecular identification assays from 1994 to 2014, with maps of relevant species distribution provided herein.

## Introduction

Malaria remains one of the most important parasitic diseases in Thailand, and has long been one of the major causes of morbidity/mortality, especially along the international borders with Cambodia, Myanmar, and Malaysia [1]. Many previous studies on malaria vector species distributions are based on morphological identification alone [2,3], including techniques on the study of morphological variations, use of cross-mating experiments, examining mitotic and meiotic karyotypes, heterochromatin variations of polytene chromosomes, electrophoretic protein variations, and the use of cuticular hydrocarbons. Unfortunately, all earlier ecological and behavioral studies based exclusively on morphologically identified specimens are questionable with regard to the precise species observed. In the past decade, more genetics and molecularbased species identification techniques have been developed and applied for the detailed study of anophelines, leading to the recognition of additional species and species complexes [4-6]. These advanced laboratory and molecular techniques have used cytogenetic/polytene chromosome compatibility and cross-mating experiments, isoenzyme

Corresponding author: Chareonviriyaphap, T. (faasthc@ku.ac.th). Keywords: Anopheles; distribution maps; Thailand. protein markers, species-specific DNA probes, PCR technologies, DNA sequencing of the ribosomal ITS2 (internal transcribed spacer) and D3 explansion segments, mitochondrial DNA (mtDNA) cytochrome oxidase II (COII) sequences, and allele-specific PCR (AS-PCR) and random amplified polymorphic DNA (RAPD) markers to discern and accurately differentiate individual species from one another [7].

The Ministry of Public Health in Thailand has classified malaria control areas based on the risk of acquiring infection (A1, A2, B1, and B2 in decreasing order) and the presence or absence of malaria vector species. The accurate identification of malaria vector species is essential to gain a better understanding of the role of each species in malaria transmission and to improve the effectiveness and proper targeting of disease control program activities. This review presents the current known distribution for all implicated (confirmed) malaria vector species in Thailand as identified by molecular methods.

## Anopheles vectors of human malaria parasites

At least 74 species of *Anopheles* mosquitoes have been documented in Thailand, comprising 73 formally named species and a closely related species of *Anopheles gigas* Giles [8,9]. Moreover, three additional species in the *An*. *barbirostris* complex are being formally named (R.E. Harbach, personal communication). Several of the most important malaria vectors in Thailand are members of closely related sibling species that may differ considerably in their biology, behavior, and epidemiological characteristics related to disease transmission, including susceptibility to malaria parasites [10] and to insecticides used to control the vector. The various sibling species show distinct distribution patterns and population densities that can often vary according to seasonal climatic changes and other factors (e.g., land use).

The most important malaria vectors are in the subgenus *Cellia*, and include members in the Leucosphyrus Group (Neomyzomyia Series), Maculatus Group (Neocellia Series), and the Minimus Subgroup (Myzomyia Series) 11–15].