

## PHYLOGENETIC RELATIONSHIPS AMONG MALARIA VECTORS AND CLOSELY RELATED SPECIES IN THAILAND USING MULTILOCUS DNA SEQUENCES

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**ABSTRACT.** The evolutionary and taxonomic status is important for understanding speciation events and phylogenetic relationships between closely related vector and nonvector species. This information is useful for targeting important disease vector species groups for the development of novel genetic-based vector and pathogen control methods. In this study, different phylogenetic analyses were performed to reconstruct phylogenetic trees for the primary malaria vectors in Thailand based on sequence information of 4 DNA fragments from the nuclear and mitochondrial regions. The primary *Anopheles* species in the subgenus *Cellia* involved in malaria transmission in Thailand separate clearly into 3 distinct clades: the Leucosphyrus group, Minimus subgroup, and Maculatus group. The phylogenetic trees based on different reconstructed algorithms and different gene regions provided congruent phylogenetic status of the mosquito species studied. The phylogenetic relationships of malaria vector species examined followed similar patterns based on morphological characters. An estimate of the divergence time among the *Anopheles* species infers that they were present during the Eocene and Miocene periods (>41 million years ago). Congruent phylogenetic analysis of malaria vectors is presented with different algorithms and gene regions. The nuclear TOLL6 fragment appears useful for molecular phylogenetic, species DNA barcode, and *Anopheles* population genetic analyses.

**KEY WORDS** *Anopheles*, *Cellia*, DNA, multilocus, phylogeny, Thailand

### INTRODUCTION

*Anopheles* mosquitoes, as vectors of pathogens of malaria and lymphatic filariasis, burden the lives of more humans than any other group of arthropods. In particular, malaria is the most significant vector-borne disease in the tropical and subtropical areas of the world (Mehlotra and Zimmerman 2006, Dixit et al. 2010). In Thailand, the threat of malaria exists throughout much of the country, and its burden is especially significant along the western (Thai–Myanmar), eastern (Thai–Cambodia), and southern (Thai–Malaysia) borders.

Approximately 539 species of *Anopheles* are currently known, and most (469) have been formally named (Harbach 2013). *Anopheles* spp. occur nearly worldwide, but far greater species diversity occurs in subtropical and tropical zones compared with temperate zones. Representatives of the genus can be found in a wide variety of different ecological and environmental conditions and from mountainous elevations to lowland, coastal zones (Sinka et al. 2010). Most phylogenetic studies of mosquitoes have dealt with species within designated groups, subgroups, and complexes, with a historical focus on *Anopheles* vectors of human malaria. Harbach (2013) remarked that none of these studies are regarded as

complete in terms of taxonomic coverage of any particular group, thus prompting further research and analysis for a greater understanding of vector systematics. Phylogenetic information is important in determining a vector capacity (i.e., probability or likelihood) to transmit disease and genetically determined mechanisms for insecticide resistance for better management of vector control programs.

In Thailand, the primary malaria vectors belong in the subgenus *Cellia*. Taxonomically, the subgenus is divided into a number of “series,” with larger series divided into species “groups,” and some groups further subdivided into “subgroups” and species “complexes.” The groupings at each level of classification are presumed to represent natural clusters of more closely related species, thus implying more proximal phylogenetic relationships than species outside the particular grouping (Harbach 2013). However, more taxonomic research is required before most of the formal and informal taxa can be firmly established as monophyletic entities (Harbach 2013).

Species in the subgenus *Cellia* are confined to the Afrotropical, Australasian, Oriental, and Palearctic biogeographical regions. In the Oriental region, which includes Thailand, representative species in the *Myzomyia*, *Neocellia*, *Neomyzomyia*, and *Pyretophorus* series are present. The *Myzomyia*, *Neocellia*, and *Pyretophorus* series are found in the Afrotropical and Oriental regions, but only species in the Minimus subgroup (*Myzomyia* series) are common to both regions (Harbach 2013). The *Myzomyia* series is a dominant grouping in Africa, where *Anopheles funestus* Giles is a principal malaria vector (Sinka et al. 2010). Related species of the

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