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MOLECULAR IDENTIFICATION OF FOUR MEMBERS OF THE *ANOPHELES DIRUS* COMPLEX USING THE MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT I GENE

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ABSTRACT. Precise mosquito species identification is an essential step for proper management and control of malaria vectors. Misidentification of members in the *Anopheles dirus* complex, some which are primary malaria vectors in Thailand and mainland Southeast Asia, remains problematic because of indistinguishable or overlapping morphological characters between sibling species. Moreover, there is a need for alternative methods, since the existing molecular techniques in the literature are not entirely satisfactory in differentiating all members in the *An. dirus* complex. The nucleotide polymorphisms in the mitochondrial cytochrome c oxidase subunit I (COI) sequences were developed to identify the 4 species within the *An. dirus* complex using an allele-specific (AS) multiplex polymerase chain reaction (PCR). The identified primers amplified and clearly differentiated the 4 members of the complex found in Thailand, *Anopheles dirus*, *An. cracens*, *An. scanloni*, and *An. baimaii* with PCR products 428/104, 236, 625, and 428 bp, respectively. These results demonstrate that an AS-PCR based on the COI region can accurately identify 4 members of *An. dirus* complex and would be useful as an alternative PCR-based method for accurate species identification.

KEY WORDS *Anopheles baimaii*, *Anopheles cracens*, *Anopheles dirus*, *Anopheles scanloni*, cytochrome c oxidase subunit I, *An. dirus* complex, multiplex polymerase chain reaction, species identification

INTRODUCTION

Currently, the *Anopheles dirus* complex contains 7 species, of which *Anopheles dirus* s.s. and *Anopheles baimaii* are implicated as important malaria vectors in Thailand and some neighboring countries in Asia. In Thailand, this complex consists of 5 isomorphic species: *Anopheles dirus* Peyton and Harrison, *Anopheles cracens* Sallum and Peyton, *Anopheles scanloni* Sallum and Peyton, *Anopheles baimaii* Sallum and Peyton, and *Anopheles nemophilous* Peyton and Ramalingam (Baimai et al. 1981, 1988a, 1988b; Sallum et al. 2005; Manguin et al. 2008). *Anopheles dirus* is the most geographically widespread species in the country, while *An. baimaii* is more limited in known distribution, occurring mainly in forested areas near the Myanmar–Thailand border and some areas of southern Thailand (Baimai et al. 1988a). *Anopheles cracens* and *An. nemophilous* are primarily found in southern Thailand extending to and beyond the Malaysian–Thai border. *Anopheles scanloni* has been identified in some areas of the south and Kanchanaburi Province in western Thailand (Poopittayasataporn and Baimai 1995). These species are commonly found in the densely forested hill environments, varying from tropical rain forests to deciduous and/or bamboo dominated environments, and into agricultural areas in certain circumstances (Baimai et al. 1988b, Oo et al. 2002, Prakash et al. 2002). *Plasmodium* transmission capacity of *An. dirus*, *An. scanloni*, and *An. baimaii* is sufficient to regard all 3 species as important malaria vectors in their respective locations. On the other hand, although *An. cracens* and *An. nemophilous* hold potential, neither species is considered a malaria vector of any significance (Peyton and Harrison 1980; Baimai et al. 1988a, 1988b; Manguin et al. 2008).

One of the key obstacles for controlling malaria transmission and developing cost-efficient, species-specific intervention strategies has been crucial errors in misidentification of sibling species members in the *An. dirus* complex using standard morphological characters alone. Given that each species can demonstrate a different site-specific role in malaria transmission, inherent phenotypic misidentification is likely to have an important impact on the planning and management of malaria vectors in specific areas (Green et al. 1990; Van Bortel et al. 1999, 2000; Phuc et al. 2003). For resolving this difficulty, molecular identification of isomorphic species has become an important tool for more accurate differentiation of primary vector species. For the *An. dirus* complex, there remains a need for alternative molecular methods because the existing techniques developed by Walton et al. (1999) and Manguin et al. (2002) are not entirely satisfactory in differentiating all members in the complex.

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