Parasites & Vectors

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A multiplex PCR assay for the identification of five species of the *Anopheles barbirostris* complex in Thailand

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Abstract

Background: The Barbirostris Complex comprises six formally described species that cannot be differentiated based on morphology alone. Out of these six species, two have been reported as putative malaria vectors, *An. campestris* and *An. wejchoochotei*. Five species are present in Thailand, *An. barbirostris*, *An. campestris*, *An. dissidens*, *An. saeungae* and *An. wejchoochotei*, while *An. vanderwulpi* occurs in Indonesia. As these species cannot be accurately differentiated by morphological characters, there is a crucial lack of information on their bionomics and role in the transmission of malaria and filariasis agents.

Results: For differentiating the six species, an allele-specific amplification (AS-PCR) based on the second internal transcribed spacer (ITS2) sequence was developed. From 862 mosquitoes in the Barbirostris Complex collected in 23 provinces throughout Thailand, the AS-PCR was able to identify five species and its validation was undertaken on 185 specimens.

Conclusions: This multiplex-PCR assay is potentially able to definitely identify all six species of the Barbirostris Complex and was validated on five species present in Thailand.

Keywords: *Anopheles*, Barbirostris Complex, ITS2, multiplex PCR, Thailand

Background

Anopheles (*Anopheles*) *barbirostris* belongs to the Barbirostris Complex within the Barbirostris Group of the Myzorhynchus Series [1]. Recently, Taai & Harbach [2] described within the Barbirostris Complex three new species, *An. dissidens*, *An. saeungae* and *An. wejchoochotei*, which accounts for six formally named species including *An. barbirostris*, *An. vanderwulpi* and *An. campestris*, the latter one being recognized as a member of this complex [2]. Four species are reported as primarily zoophilic throughout their geographic range, although they may bite humans in the absence of their usual hosts (typically bovids). The two others, *An. wejchoochotei* and *An. campestris*, are known for their greater anthropophilic behavior, especially the latter species that more readily bites humans than any other members of the Barbirostris Complex [2, 3]. *Anopheles barbirostris* (*s.l.*) is widely distributed in Thailand [4, 5] and more globally in the Asian region [2, 6–8]. It has been reported as a vector of *Plasmodium falciparum* and *Plasmodium vivax* in Sri Lanka, Bangladesh, Indonesia (Sumatra, Sulawesi, Flores), Timor Leste, as well as a secondary vector on the island of Borneo [9] and a putative malaria vector in the Aranyaprathet District, Sa Kaeo Province, southeastern Thailand [10, 11]. More specifically, *An. barbirostris* (*s.l.*), *An. campestris* and *An. wejchoochotei* (former '*campestris*-like', see Table 1) have been incriminated as vectors of *P. falciparum* and *P. vivax* [2–4, 11–17]. However, the lack of reliable methods to identify the species within the complex has hampered precise evaluation of the specific role of each member in transmission of malaria and other pathogens, e.g. *Brugia timori* and *Brugia malayi* in Indonesia [12, 29].

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