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Species diversity and insecticide resistance within the *Anopheles hyrcanus* group in Ubon Ratchathani Province, Thailand

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Abstract

Background: Members of the *Anopheles hyrcanus* group have been incriminated as important malaria vectors. This study aims to identify the species and explore the insecticide susceptibility profile within the *Anopheles hyrcanus* group in Ubon Ratchathani Province, northeastern Thailand where increasing numbers of malaria cases were reported in 2014.

Methods: Between 2013 and 2015, five rounds of mosquito collections were conducted using human landing and cattle bait techniques during both the rainy and dry seasons. *Anopheles* mosquitoes were morphologically identified and their insecticide susceptibility status was investigated. Synergist bioassays were carried out with *An. hyrcanus* (*s.l.*) due to their resistance to all insecticides. An ITS2-PCR assay was conducted to identify to species the Hyrcanus group specimens.

Results: Out of 10,361 *Anopheles* females collected, representing 18 taxa in 2 subgenera, 71.8% were morphologically identified as belonging to the Hyrcanus Group (subgenus *Anopheles*), followed by *An. barbirostris* group (7.9%), *An. nivipes* (6.5%), *An. philippinensis* (5.9%) and the other 14 *Anopheles* species. Specimens of the Hyrcanus Group were more prevalent during the rainy season and were found to be highly zoophilic. *Anopheles hyrcanus* (*s.l.*) was active throughout the night, with an early peak of activity between 18:00 h and 21:00 h. ITS2-PCR assay conducted on 603 DNA samples from specimens within the Hyrcanus Group showed the presence of five sisters species. *Anopheles peditaeniatus* was the most abundant species (90.5%, $n = 546$), followed by *An. nitidus* (4.5%, $n = 27$), *An. nigerrimus* (4.3%, $n = 26$), *An. argyropus* (0.5%, $n = 3$), and *An. sinensis* (0.2%, $n = 1$). All *An. hyrcanus* (*s.l.*) specimens that were found resistant to insecticides (deltamethrin 0.05%, permethrin 0.75% and DDT 4% and synergist tests) belonged to *An. peditaeniatus*. The degree of resistance in *An. peditaeniatus* to each of these three insecticides was approximately 50%. Addition of PBO (Piperonyl butoxide), but not DEF (S.S.S-tributyl phosphotritioate), seemed to restore susceptibility, indicating a potential role of oxidases as a detoxifying enzyme resistance mechanism.

Conclusions: A better understanding of mosquito diversity related to host preference, biting activity and insecticide resistance status will facilitate the implementation of locally adapted vector control strategies.

Keywords: *Anopheles hyrcanus*, Malaria vectors, Species diversity, Insecticide resistance, Ubon Ratchathani Province, Thailand

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