THE *ANOPHELES CULICIFACIES* SPECIES COMPLEX IN RELATION TO MALARIA TRANSMISSION IN SRI LANKA

A THESIS PRESENTED BY

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Abstract

Anopheles culicifacies Giles *sensu lato* (Diptera: Culicidae) is the predominant vector of malaria in Sri Lanka. Until recently, only sibling species B was reported to be present in Sri Lanka. The conundrum was that species B was reportedly a poor vector of malaria in India. The present study resolves this issue by demonstrating for the first time that what was described as species B in Sri Lanka is really a mixture of two species, *viz.* B and E, that are also apparently present in Rameshwaram island, India. Species B and E share the same polytene chromosome configuration. However they differ in that the mitotic Y-chromosome is acrocentric in species B and submetacentric in species E.

Differences between *An. culicifacies* species B and E can impact on malaria control measures undertaken in the country. Entomological parameters of species B and E, such as fecundity, longevity, susceptibility to infection by human malaria parasites, and insecticide resistance, that influence vector potential and the dynamics of malaria transmission are described here for the first time.

Egg production determines vector numbers that is in turn directly related to the rate of spread of malaria. The fecundity of the two sibling species though slightly higher for species E, was not significantly different in laboratory studies.

The capacity of the vector to survive long enough to support the complete development of the malaria parasites is exponentially related to the rate of malaria transmission. This was estimated through measuring the number of dilatations in the ovarioles in field-caught female *An. culicifacies*. The results showed that species E females have significantly greater longevity and therefore a greater potential to spread malaria.

Susceptibility to insecticides used in malaria control influences the survival of infected and uninfected mosquitoes thereby affecting the malaria transmission rate. Both species B and E were completely susceptible to 0.05% lambdacyhalothrin and 0.05% deltamethrin, while being totally resistant to 4% DDT, in standard WHO tests. However Species B was however significantly more susceptible to malathion than species E. The cause of the different sensitivity to malathion was not established in the present studies, but it may reflect genetic factors and/or the greater exposure of species E to malathion and other organophosphates used for agricultural purposes.

The study also showed that species E can support the extrinsic cycle of *Plasmodium vivax* and *P. falciparum* at least to the stage of oocyst formation in the midgut. However none of the limited number of female species B fed on blood from the same *P. vivax* or *P. falciparum* gametocyte carriers developed infections. Statistical analysis showed a significant association between sibling species and the formation of *P. vivax* oocysts in the midguts. The results confirm that species E can

be infected with *P. vivax* and *P. falciparum*. The vectorial capacity of species B is low or nil in Sri Lanka, as it is also the case in India.

A DNA based technique is needed to circumvent the time-consuming and labour-intensive cytogenetic technique which is the only one presently available to distinguish *An. culicifacies* species B and E. For this purpose a partial sequence of the relevant portion of the cytochrome oxidase II (COII) gene from karyotyped B and E Sri Lankan specimens was obtained. On aligning with the sequences of Indian species B and E that reportedly possess species-specific residues, no correlation between the sequences of species B and E from India and Sri Lanka was found. Both Sri Lankan species B and E shared the same sequence at 4 of the 5 reportedly variable sites in the Indian COII. Thus the sequence of this fragment is not useful for distinguishing the two sibling species in Sri Lanka. Analysis of available sequence data of the internal transcribed spacer 2 (ITS2) including partial sequences for the 5.8S and 28S ribosomal RNA genes also showed no sequence variation between species B and E of Sri Lanka. Both Sri Lankan ITS2 sequences matched that reported for Indian species E and not Indian species B. Hence other DNA-based approaches need to be developed to distinguish species B and E in Sri Lanka.